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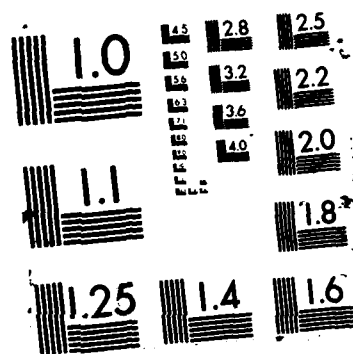
ENTROPIC ELASTIC PROCESSES IN PROTEIN MECHANISMS PART 2  
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ENTROPIC ELASTIC PROCESSES IN PROTEIN MECHANISMS

PART 2. SIMPLE (PASSIVE) AND COUPLED (ACTIVE) DEVELOPMENT OF ELASTIC FORCES

Dan W. Urry

Laboratory of Molecular Biophysics  
The University of Alabama at Birmingham  
P. O. Box 311/University Station  
Birmingham, Alabama 35294

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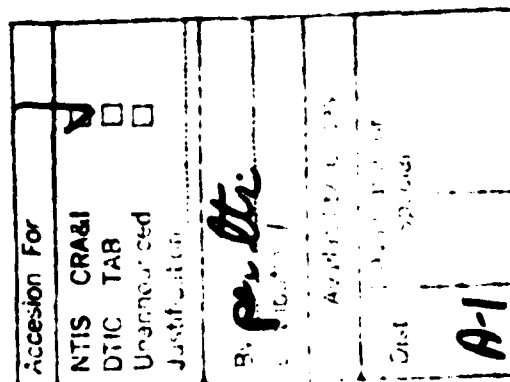
"At every crossway on the road that leads to the future,  
tradition, has placed against each of us, 10,000 men to guard the pass."  
Maurice Maeterlinck, 1907

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ABSTRACT	3
I. INTRODUCTION	4
II. ELASTIC PROCESSES IN PROTEIN SYSTEMS: CLASS I	6
A. Elastin	6
1. Elastogenesis	
2. Characterization of the Elasticity of Elastin	
B. The Elastic (Third) Filament of Muscle	9
C. Entropic Elastomeric Force in Enzyme Catalysis	11
D. Entropic Elastomeric Force in Channel Conductance State Changes	13
III. OXIDATIVE PROCESSES ALTERING STRUCTURE AND ELASTIC FUNCTION	14
A. Effect of Prolyl Hydroxylation on Fiber Formation	14
B. Oxidative Loss of Elastic Recoil	16
C. Relevance to Environmentally Induced Lung Disease	17
IV. CHEMICAL MODULATION OF ELASTIC FORCES: CLASS II	18
A. Elastic Molecular Machines and a Motive Force in Protein Mechanisms	20
1. Using Temperature Change to Turn "On" and "Off" Elastomeric Force	
2. Using a Change in pH to Turn "Off" and "On" Elastomeric Force	
3. Possible Chemical Processes at Constant pH and Temperature	
a. Deamidation/Amidation as an On/Off Switch	
b. Dephosphorylation/Phosphorylation as an On/Off Switch	
c. Changes in Calcium Ion Activity as an On/Off Switch	
B. Events Attending Parturition and their Reversal	24
1. Interpubic Ligament Formation and Reversal	
2. Cervical Ripening	
C. Muscle Contraction	27
D. Cell Motility	30



## ABSTRACT

In the first part of this review on entropic elastic processes in protein mechanisms (Urry, 1987), it was demonstrated with the polypentapeptide of elastin,  $(\text{Val}^1\text{-Pro}^2\text{-Gly}^3\text{-Val}^4\text{-Gly}^5)_n$ , that elastic structure develops as the result of an inverse temperature transition and that entropic elasticity is due to internal chain dynamics in a regular non-random structure. This demonstration is contrary to the pervasive perspective of entropic protein elasticity of the past three decades wherein a network of random chains has been considered to be the necessary structural consequence of the occurrence of dominantly entropic elastomeric force. That this is not the case provides a new opportunity for understanding the occurrence and the role of entropic elastic processes in protein mechanisms.

Entropic elastic processes are considered in two classes: passive and active. The development of elastomeric force on deformation is Class I (passive) and the development of elastomeric force as the result of a chemical process shifting the temperature of a transition is Class II (active). Examples of Class I are elastin, the elastic filament of muscle, elastic force changes in enzyme catalysis resulting from binding processes and resulting in the straining of a scissile bond, and in the turning on and off of channels due to changes in transmembrane potential. Demonstration of the consequences of elastomeric force developing as the result of an inverse temperature transition are seen in elastin where elastic recoil is lost on oxidation, i.e., on decreasing the hydrophobicity of the chain and shifting the temperature for the development of elastomeric force to temperatures greater than physiological. This is relevant in general to loss of elasticity on aging and more specifically to the development of pulmonary emphysema.

Since random chain networks are not the products of inverse temperature transitions and since the temperature at which an inverse temperature transition occurs depends on the hydrophobicity of the polypeptide chain, it now becomes possible to consider chemical processes for turning elastomeric force on and off by reversibly changing the hydrophobicity of the polypeptide chain. This is herein called mechanochemical coupling of the first kind and this is the chemical modulation of the temperature for the transition from a less-ordered, less elastic state to a more-ordered, more elastic state. In the usual considerations to date, development of elastomeric force is the result of a standard transition from a more-ordered, less elastic state to a less-ordered, more elastic state. When this is chemically modulated, it is herein called mechanochemical coupling of the second kind. For elastin and the polypentapeptide of elastin, since entropic elastomeric force results on formation of a regular non-random structure and since thermal randomization of chains results in loss of elastic modulus to levels of limited use in protein mechanisms, consideration of regular spiral-like structures rather than random chain networks or random coils are proposed for mechanochemical coupling of the second kind. Chemical processes to effect mechanochemical coupling in biological systems are most obviously phosphorylation/dephosphorylation and changes in calcium ion activity but also changes in pH. These issues are considered in the events attending parturition, in muscle contraction and in cell motility.

## I. INTRODUCTION

Elastic processes in proteins may be considered as one of two classes: Class I, passive and simple elastic processes, those demonstrated when the protein is acted upon by an external force with the result of substantial deformation, and Class II, active and coupled elastic processes, those resulting for example from a change in chemical potential which effects transition between two structural states, one of which can exert an elastomeric force. Class II includes interesting cases of mechanochemical coupling. Examples of Class I involve the obvious deformations that occur when a joint flexes stretching ligaments and overlying skin, when lung is expanded and when a pressure pulse passes through a blood vessel. The resting tension of muscle, particularly when the muscle is pulled beyond the overlap of thick and thin filaments, may be considered an example of the first class. Elastic deformation can also be considered to result from binding processes as for example in the binding of a substrate to an enzyme by the induced fit mechanism of enzyme catalysis and in the binding of allosteric effectors; both could manipulate elastic forces in a manner that would reduce the activation energy for the catalytic event. Deformations of membrane proteins due to the presence of an electric field across the membrane could occur and an example would involve the mechanism for opening and closing of ion selective transmembrane channels.

Most dramatic examples of active elastic processes (Class II) would be the onset of elastic forces in muscle contraction and in cell motility in general. The essential element of the concept outlined here is the transition to an entropic elastic state at a fixed temperature by means of a change in chemical potential where the change in chemical potential changes the temperature of a transition between two states with, for example, the higher temperature state being the entropic elastic state. The transition can be an inverse temperature transition (from higher to lower chain entropy on increasing temperature) in

which case a change in the hydrophobicity of the chain segment effects the shift in midpoint of the transition temperature; this can be considered mechanochemical coupling of the first kind. Alternatively, the transition can be from a low entropy state to a higher entropy state in which case chemical destabilization of the lower entropy state or stabilization of the higher entropy state can shift the temperature of the transition. This is what has generally been considered for mechanochemical coupling; it will here be referred to as mechanochemical coupling of the second kind. Mechanochemical coupling therefore is taken to be a chemical process converting one structural state to second state capable of exerting an entropic elastomeric force. In order most effectively to provide an entropic motive force, the second elastic state should be energetically preferred when in the extended state such that while extended, an entropic elastomeric force would result in an elastic recoil to a less extended state. Under these circumstances, entropic elastomeric force would be particularly favorable rather than an internal energy component of elastomeric force.

Elastic processes of the first class will be considered in regard to elastin, to the third (elastic) filament of muscle, to enzyme catalysis and to conductance state changes in channels. With respect to elastin, oxidative processes will be discussed which alter structure and elastic function with relevance to wound repair and to environmentally induced lung disease. Finally, elastic processes of the second class which involve the reversible turning "on" and "off" of elastic forces at a fixed temperature will be considered in relation to synthetic elastomers, to events attending parturition and their reversal, to muscle contraction and to cell migration in a concentration gradient. The approach to the additional biological elastic processes will follow the inquiry as to what properties of, and concepts derived from, the elastin system might be relevant to the observed properties of the additional systems where elastic forces are involved.

## II. ELASTIC PROCESSES IN PROTEIN SYSTEMS: CLASS I

### A. Elastin

For the past three decades, the dominant perspective of the nature of protein elasticity as represented by elastin has been that of the classical theory of rubber elasticity (Hoeve and Flory, 1958). This perspective was reaffirmed in 1974 with the summarizing statement that "A network of random chains within the elastin fibers, like that in a typical rubber, is clearly indicated" (Hoeve and Flory, 1974). In what follows, the random chain network perspective will be shown to be incorrect and with a new perspective come new insights into elastic processes in protein function and dysfunction. The most striking primary structural feature of elastin is the repeating pentapeptide sequence characterized in PART 1 (Urry, 1987); its prominence in the primary structure of elastin is shown in Figure 1 (Sandberg, et al., 1981, 1985; Indik, et al., in press) where the primary structure is listed in terms of the sequences between cross-linking lysine residues. It was shown in PART 1 (Urry, 1987) that cross-linked high polymers of the repeating pentamer sequence develop elastomeric force in concert with the occurrence of an inverse temperature transition and that the entropically elastic cross-linked polymers lose elastomeric force by means of thermal denaturation. Of course networks of random chains are not the products of inverse temperature transitions and networks of random chains do not exhibit thermal denaturation. In this section, it will be demonstrated that elastin also develops elastomeric force by means of an inverse temperature transition and loses elastomeric force by means of thermal denaturation.

#### 1. Elastogenesis

The precursor protein of elastin, tropoelastin (Smith, et al., 1968; Sandberg, et al., 1969; Smith, et al., 1972), and a 70,000 mw chemical fragmentation product of elastin,  $\alpha$ -elastin (Partridge, et al., 1955; Partridge



and Davis, 1955), are each soluble in water below 20°C. When the temperature is raised, tropoelastin and  $\alpha$ -elastin aggregate as shown for the polypentapeptide of elastin in PART 1 (Urry, 1987). When drops of these cloudy solutions are placed on a carbon coated grid and similarly stained, these molecules randomly dispersed in solution at lower temperature are seen to have self-assembled in the formation of parallel-aligned filaments as shown in Figure 2 (Cox, et. al, 1973 and 1974). Optical diffraction of the micrographs demonstrate 5 nm periodicities (Volpin, et al., 1976). Thus elastogenesis is the result of an inverse temperature transition, just as polypentapeptide fibrillogenesis is the result of an inverse temperature transition. Circular dichroism data of solution and coacervates of  $\alpha$ -elastin similarly show an increase in intramolecular order with increase in temperature (Starcher, et al., 1973).

## 2. Characterization of the Elasticity of Elastin

When a sample of ligamentum nuchae elastin is stretched to 60% extension at 40°C and then the temperature dependence of elastomeric force is determined, there is a steep development of elastomeric force in the 20° to 40°C temperature range (see Figure 3). This is analogous to that of the polypentapeptide of elastin though the transition is not as sharp. In the 50° to 60°C temperature range when the data is plotted as  $\ln[\text{force/temperature}(\text{°K})]$  versus temperature, the slope is near zero. If the study is carried out in 30% ethylene glycol and 70% water as done by Hoeve and Flory (1958), the rise in elastomeric force is shifted to lower temperature and the temperature range, in which the near zero slope is obtained, is wider (unpublished data). This is the solvent system on which Hoeve and Flory (1958, 1974) concluded that elastin was dominantly an entropic elastomer. The result is obtained when the temperature is raised relatively fast, taking only 20 to 30 minutes per 4°C from 40°C to 75°C. If, however, the data is obtained at a rate of four hours per four degree

rise in temperature as subsequently done in Figure 3, there is observed an irreversible loss of elastomeric force above 55°C. This is thermal denaturation as can also be shown by determining stress/strain curves with interceding 24 hour periods of heating at 80°C and 0% extension, as shown in Figure 4. When a plot of  $\ln(\text{elastic modulus})$  is plotted against time of heating at 80°C, a half-life for the thermal denaturation is obtained. The half-life at 80°C is long, about ten days, as may be obtained from the insert of Figure 4. Thus elastin can also be observed to develop elastomeric force as the result of an inverse temperature transition and to lose elastomeric force due to thermal denaturation. These are not the properties of random chain networks. In fact, the randomized networks resulting from thermal denaturation exhibit significantly reduced elastic moduli. If thermal randomization of polypeptide chains in ligamentum nuchae elastin is representative, then with the number of cross-links no more than in elastin, the elastic moduli of random polypeptide chains would seem to be of limited utility to many elastic processes in proteins.

To further characterize the nature of the elasticity of elastin the temperature dependence of length under zero load has been determined (Urry, et al., 1986) and elastin is found to exhibit lengthening on lowering the temperature as observed for the polypentapeptide but unlike the results for latex rubber (see Figure 13 of PART 1). Furthermore, a dielectric relaxation study has been carried out on the coacervate of  $\alpha$ -elastin (Urry, et al., 1985). As shown in Figure 5,  $\alpha$ -elastin exhibits a dielectric relaxation spectrum in the 1 MHz to 1 GHz frequency range that is similar to that of the polypentapeptide of elastin shown in Figure 12 of PART 1. The insert of Figure 5 shows the temperature dependence of correlation time from which the energy barrier to mobility is approximated to be 1.5 kcal/mole (Urry, et al., 1985). This is the same value obtained for the backbone mobility of the polypentapeptide of elastin, and

interestingly, it is essentially the same value as obtained by Aaron and Gosline (1980 and 1981) for single elastic fibers from optical anisotropy studies. Accordingly, even in this brief summary, the data demonstrate elastin to exhibit dominantly entropic elastomeric force but to do so by means of a non-random chain network. These results become central to understanding elastin function and pathology (see part III below).

#### B. The Elastic (Third) Filament of Muscle

The third most abundant protein in skeletal muscle (Wang, 1985) after myosin of the thick filament and actin of the thin filament is a megadalton elastic protein called connectin by Maruyama (Maruyama, et al., 1976, 1977) and titin by Wang (Wang and Ramirez-Mitchell, 1979 and Wang, et al., 1979). In the electron microscope connectin(titin) can be observed as long, slender filaments (see Figure 6). This protein is now considered to be the source of the "gap filaments" so apparent in insect flight muscle (Trombitas and Tigy-Sebes, 1974). In 1974, A. F. Huxley noted "Additional filaments, which are seen when the myosin is dissolved away or when the muscle is stretched so far that there is a gap between the thick and thin filaments, are not shown because it is not yet known where they attach to the other structures in the filament array," (Huxley, 1974). The titin protein has now been identified by Wang and coworkers (Wang, personal communication) to form a filament which spans from the Z-line to the M-line with evidence that a single polypeptide chain of  $1$  to  $2 \times 10^4$  residues spans the total distance of about  $1.2 \mu\text{m}$  ( $1.2 \times 10^4 \text{ \AA}$ ). It is thought to be responsible for the resting tension of muscle and to contribute to the tension in stretched muscle fibers when the thick and thin filaments have slid past each other to beyond overlap (Magid, et al., 1984). Titin has now been observed in vertebrate and invertebrate skeletal and cardiac muscle (Wang, 1985). A representation of the three filament model of the sarcomere is shown in Figure 7 (Magid, et al., 1984).

Information on the nature of the elasticity of this protein has been provided by Maruyama, et al. (1977), by demonstrating that the temperature coefficient of tension is positive and that this is the case at all extensions examined. This is a statement of the second term,  $T(\partial S/\partial L)$ , of Equation 2 in PART 1, that is, this protein exhibits entropic elastomeric force. A megadalton protein that can be observed as long, slender filaments and which is thought to span a 1.2  $\mu\text{m}$  length is not readily characterizable as having a random distribution of end-to-end lengths as required by the classical theory of rubber elasticity. It seems therefore that it is more readily described in terms of spiral-type structures with analogy to the  $\beta$ -spiral of the polypentapeptide of elastin. The molecular weight and the distance spanned by a single chain means that there are 1 to  $2 \times 10^4$  residues spanning a distance of as many Angstroms with from 1.0 to 0.5  $\text{\AA}$ /residue. This is the range of the number of Angstroms/residue along the spiral axis of the  $\beta$ -spiral of the polypentapeptide of elastin. Interestingly, "titin contains an inordinate amount of proline (8 - 9%)" (Wang, private communication) and there is a similar amount of glycine (Wang, 1985). While not a requirement for a librational entropy mechanism of elasticity, this allows the possibility of the occurrence of  $\text{Pro}^2\text{-Gly}^3$  Type II  $\beta$ -turns. The circular dichroism data so far reported are consistent with this possibility (Trinick, et al., 1984; Maruyama, et al., 1986). Interestingly, a dielectric relaxation spectrum of muscle (see Figure 8; Grant, et al., 1978; Schwan, 1974) demonstrates relaxations in the same frequency range as observed for elastin in Figure 5 and for the polypentapeptide in Figure 12 in PART 1. There are additional features in the amino acid composition that are of relevance to mechanochemical coupling of the first kind to be discussed below (see part IV below). Some 12 - 15% of the residues are serines and threonines; this with an equivalent amount of lysines and arginines raises the possibility for kinase sites,

i.e., sites for phosphorylation. Indeed purified rabbit titin is reported to contain 2 - 3 moles of phosphate bound per mole of protein (Somerville and Wang, 1983). As will be discussed below, the effects of phosphorylation/dephosphorylation provide means of turning "off" and "on" elastomeric force in mechanochemical coupling of the first kind by increasing/decreasing the hydrophilicity with a resulting increase/decrease in length of the chain (see part IV below).

### C. Entropic Elastomeric Force in Enzyme Catalysis

In discussion of the separation of an observed elastomeric force into its internal energy and entropy components in part IIA of the preceding article (PART 1; Urry, 1987), it was apparent that an entropic component of force would be just as effective in inducing bond strain as an internal energy component of force which is due to bond strain. This becomes of interest in the concepts of elastomeric force inducing strain in enzyme catalysis (Lumry and Eyring, 1954; Lumry and Gregory, 1986) particularly when considered in the induced fit mechanism of Koshland (1963). Furthermore as noted in part IVA of the first article (Urry, 1987) a peptide segment of but a few residues by the librational entropy mechanism could exhibit significant entropic elastomeric force. Because of data and analysis already available, carboxypeptidase A provides an interesting example to consider in these regards. Using the following form of the Kramers (1940) expression

$$k_{cat} = \frac{1}{\tau_x} \exp \left( -\frac{\Delta U}{RT} \right) \quad (10)$$

where  $k_{cat}$  is the initial rate of enzyme catalysis;  $\tau_x$  is the structural relaxation time relevant to the catalytic event and  $\Delta U$  is the potential energy barrier for the reaction, Gavish (1986) reported a plot of  $\ln k_{cat}$  versus  $\ln(\text{viscosity of the medium at constant temperature})$  and determined  $1/\tau_x$  for carboxypeptidase A hydrolysis of a tripeptide to be  $7.5 \times 10^7/\text{sec}$ . Interestingly, this is a  $\tau_x$  of

13 nsec which is similar to the relaxation that develops as elastin and the polypentapeptide of elastin develop their regular, highly elastic states. (See Figures 5 above and Figure 12 of PART 1.) It is also in the range of the ultrasonic absorption maximum of proteins (Barnes, et al., 1985; Pethig, 1979; Zana and Tondre, 1972; Schneider, et al., 1969; Cho, et al., 1985). Interestingly an increase in ultrasonic absorption has been reported on conversion from zymogen to active enzyme (Cerf, 1985). In Figure 9 is a depiction due to Gavish (1986) which is to demonstrate that "... the protein is capable of straining the substrate in a highly oriented way ...", that is, the elastic forces used need to be anisotropic whereas random chain networks or random coils are isotropic.

Continuing with consideration of carboxypeptidase A and using the crystal structures of the enzyme free and when complexed with Gly-Tyr (Lipscomb, et al., 1968, Lipscomb, 1980 and Rees, et al., 1980, 1981), analysis of the atom locations by Leibman, et al. (1985) shows the major difference in a linear distance plot to involve residues 272 and 273. These residues are contiguous with the catalytically important residue Glu-270 in which, in one possible mechanism, the carboxylate forms an intermediate mixed anhydride with the carbonyl carbon of the scissile bond. The results appear consistent with a perturbation of the peptide segment that is contiguous with a functional group having critical electrostatic interactions with one side of the scissile bond. Figure 10 represents an attempt to depict this process from the standpoint of the librational entropy mechanism of elasticity in combination with the induced fit mechanism of Koshland (1963). On binding of the substrate to the enzyme, there is induced a conformational change in the enzyme which arises from the formation of favorable electrostatic and possibly hydrophobic interactions. In the process of the induced fit, a peptide segment, schematically represented as a spiral, becomes stretched. The essential element is that there occur on binding

a damping of librations with but a small extension in a peptide segment contiguous with an electrostatic interaction at one side of the scissile bond. The damping, which could involve only a few residues and the side chain at the active site and need not involve the schematic spiral structure of Figure 10, results in an entropic elastomeric force. Through the electrostatic attachment of the active site side chain, the entropic elastomeric force due to damping of motion in the polypeptide backbone and the active site side chain exerts a strain on the scissile bond. A random chain network is not required; the entropic elastic force can be derived from a short peptide segment and can arise in an oriented, anisotropic manner. To have an entropic elastomeric force induce a strain would be an effective means of reducing the internal energy or potential energy barrier for bond cleavage.

#### D. Entropic Elastomeric Force in Channel Conductance State Changes

Rubinson (1986) has recently carried out an analysis of the closed channel - open channel equilibrium for the sodium channel of nerve that is relevant to elastic processes in protein mechanisms. He considered the probability of the open state as a function of transmembrane potential. The system was modelled as charge moving in an electric field while tethered to a peptide chain segment. The force constant for the charge confined by an harmonic potential was determined to be approximately 20 dyne/cm and was used to estimate an elastic modulus. The value for the elastic modulus is obtained on assuming a value for the ratio of cross-sectional area to length for the elastomeric segment. Using 400Å for this ratio, the elastic modulus would be  $5 \times 10^6$  dyne/cm<sup>2</sup>, essentially the value for elastin. The results suggest the importance of an equilibrium between two states in an electric field in which interconversion involves the stretching of an elastic peptide segment. Below the effect of phosphorylation/dephosphorylation in modulating the elastic forces is considered and this could be important in modulation of channel state.

### III. OXIDATIVE PROCESSES ALTERING STRUCTURE AND ELASTIC FUNCTION

It has been shown above that both the development of elastin fibers and the development of elastomeric force are the result of an inverse temperature transition. As shown in Figure 14 of PART 1 (Urry, 1987), when the hydrophobicity of the polypeptide is changed, the temperature for association to form fibers changes and the temperature at which intramolecular order develops to produce the highly elastic state also changes. These changes occur in an entirely predictable way. When the hydrophobicity is increased, the transitions occur at lower temperature; when the hydrophobicity is decreased, which is equivalent to an increase in hydrophilicity (i.e., to making the polypeptide chain more polar), the transitions occur at higher temperature. This realization is fundamental to understanding function and pathology of elastin as is demonstrated below.

#### A. Effect of Prolyl Hydroxylation on Fiber Formation

One means of increasing the hydrophilicity of tropoelastin, the precursor protein of elastin, occurs naturally. The means is prolyl hydroxylation by the enzyme prolyl hydroxylase. This enzyme is essential to the formation of functional collagen; it performs a post-translational modification of collagen that is important for collagen release from the cell (Prockop, et al., 1976), that stabilizes the triple stranded collagen helix (Uitto, Hoffmann and Prockop, 1976; Berg and Prockop, 1973; Rosenbloom, et al., 1973; Ramachandran, et al., 1973; Ramachandran, et al., 1975) and that makes collagen resistant to non-specific proteolytic degradation (Berg and Prockop, 1973). The same enzyme can hydroxylate prolyl residues of tropoelastin (Uitto, et al., 1976; Sandberg, 1976; Rucker and Tinker, 1977) with quite different consequences. Using the PPP of elastin as the model system, it has been shown by means of chemical synthesis that the temperature for aggregation of  $(\text{Val}^1\text{-Hyp}^2\text{-Gly}^3\text{-Val}^4\text{-Gly}^5)_n$  is above



60°C (Urry, et al., 1979); with just 10% of the prolyl residues replaced by hydroxyproline (Hyp), the temperature for aggregation is shifted 7°C to higher temperature (see Figure 11). The action of prolyl hydroxylase on the polypeptide of elastin results in only about 1% hydroxylation (Bhatnagar, et al., 1978) and yet increases the temperature equivalent to 10% hydroxylation achieved chemically. This enhanced effect of the enzyme in shifting the temperature of the transition may be due to the distribution of Hyp along the polypeptide or possibly because the enzymatic process results in a single enantiomer, whereas in the unresolved chemically prepared Hyp the two enantiomers could be incorporated more-nearly equivalently in the peptide synthesis. Thus hydroxylation of proline, increasing hydrophilicity of the polypeptide, impairs fiber formation. This was therefore proposed to be the reason for impaired elastic fiber under conditions of a repair response as in wound repair and pulmonary fibrosis (Urry, et al., 1980). In the case of wound repair, this information provides for an understanding of why elastin fibers are so sparse in scar tissue. Quoting from the volume entitled "Wound Repair" by Peacock (1984), "In a scar, where there are few elastic fibers and where collagen fibers become oriented primarily along lines of tension, there is little 'give,' and stretching and relaxation are not possible. This accounts for rigidity of scar tissue and inability to undergo repeated deformation and recovery as needed in skin covering a joint or other moving part. Failure to include new elastic fibers in repair tissue until long after collagen fibers are formed is another example of the inferiority of scar tissue to normal tissue and has obvious implications in the repair of skin defects, ligamentous structures, and large arteries." The repair process appears to have evolved for quick wound closure at the expense of quality, possibly due to the need to limit the opportunity for infection. With the antibiotics now available, quality can become of primary concern. As an understand-

ing of elastogenesis unfolds, such as the identification of chemotactic peptides that recruit fibroblasts which have already differentiated for elastin synthesis (Senior et al., 1984), it is hoped that the quality of wound repair can be improved.

That interference with fiber formation results from high activities of prolyl hydroxylase has recently been demonstrated in cell culture by Franzblau and coworkers (Barone, et al., 1985). Prolyl hydroxylase requires ascorbate as a cofactor. This group has made the important finding that ascorbate treatment of neonatal rat aortic smooth muscle cells in culture results in overhydroxylation of elastin, results in increase in the solubility of the precursor protein at 37°C, and results in a decrease in the formation of insoluble elastin, i.e., a decrease in fiber formation. Therefore, simple extrapolation from the demonstration that elastin forms by means of an inverse temperature transition has significant biological consequences. This is even more dramatically demonstrated in what follows.

#### B. Oxidative Loss of Elastic Recoil

It is a simple prediction of our understanding of the nature of the entropic elasticity of elastin that increasing the hydrophilicity (decreasing hydrophobicity) would raise the temperature of the inverse temperature transition. The result at 37°C would be for the  $\beta$ -spiral-like structures to unwind with the resultant loss of elastomeric force, particularly when the degree of extension is limited. This prediction derives from the data in Figure 14 of PART 1 (Urry, 1987). Any oxidative process (increase in hydrophilicity) should result in a loss of elastic recoil exhibited by elastin. This perspective can be tested by means of a superoxide generating system. When a series of stress/strain curves is determined on ligamentum nuchae elastin over a period of hours during which superoxide is enzymatically released to the bathing solution, the

elastic modulus is seen to decrease progressively and the percentage extension required before a resistance is encountered increases with time. The stress/strain curves are shown in Figure 12. A plot of the  $\ln(\text{elastic modulus})$  versus time (see insert of Figure 12B) indicates a half-life for the loss of elastic modulus of about one-half day. This can be compared to the rate of thermal denaturation at 80°C in Figure 4 above where a half-life of about 10 days is observed. Oxidative processes are a potent means of destructuring elastin! Observation along the x-axis of the stress/strain curves as a function of time in Figure 12 shows the time dependence of lengthening (of unwinding). With increased time of exposure to superoxide progressively greater extensions are required before elastin resists extension. Therefore, when elastin becomes oxidized, i.e. more hydrophilic, the structures developed by means of an inverse temperature transition (that is normally largely complete by 37°C simply unwind because the increase in hydrophilicity shifts the inverse temperature transition to higher temperatures. This simple understanding of pathology provides an explanation for the loss of elastic recoil with age, as for example in the sagging and wrinkling of skin. Slowing oxidative processes becomes an obvious intervention. The insights also provide a new molecular basis for the understanding of environmentally induced lung disease (see below).

### C. Relevance to Environmentally Induced Lung Disease

In the progressive chronic disorder pulmonary emphysema, the elastic fibers become fragmented and dysfunctional with a primary symptom being loss of elastic recoil (Clark, et al., 1983). In an effort to understand this elastic fiber dysfunction, an animal model for pulmonary emphysema has been developed in which the initial insult is instillation of elastase directly into the lung (Kuhn, et al., 1976; Osman, et al., 1985b). This has supported the concept that a lack of proteinase inhibitors, which would otherwise block proteolytic degra-

dation, might be responsible (Snider, 1984; Janoff, 1985; Stone, 1983). In examining the causes of decreased antiprotease activity, studies have been carried out which demonstrate that  $\alpha$ -1-proteinase inhibitor is damaged by superoxide; increased proteolytic activity would be a consequence. With the above demonstration of the loss of elastic recoil of elastin resulting directly from oxidative processes, this becomes a reasonable primary event with a possible scenario that the action of proteolytic enzyme activity could even be part of a repair response that is not necessarily harmful. Elastolytic removal of the oxidized chains and replacement by new elastin while the elastic fiber is still present as a site of aggregation and nucleus for fiber growth would seem to be a potential remedial process. With the demonstrated oxidative loss of elastic recoil in Figure 12, it would appear that attention should be directed toward the sources of oxidants, for example in tobacco smoke (Osman, et al., 1985a), and the natural and prophylactic defensive mechanisms that may be relevant.

#### IV. CHEMICAL MODULATION OF ELASTIC FORCES: CLASS II

In the above consideration of elastic processes in protein mechanisms (part II), passive elastic processes (Class I) were considered. In this section are considered the interesting active elastic processes, examples of Class II. In the active elastic process, the protein is of a design whereby elastic forces may be turned "on" or "off" by a reversible change in a chemical element related to the system. This is mechanochemical coupling. For example, there can be a change in the activity of a chemical species such as a change in the pH or a change in the concentration of calcium ion, a  $\Delta pCa$ , or there could be a change in the hydrophilicity of the system by phosphorylation or dephosphorylation. In Figure 13 are represented structural changes for the two kinds of mechanochemical coupling. The first kind involves an inverse temperature transition. As

has been demonstrated in the foregoing cases, a change in the hydrophobicity or hydrophilicity can change the temperature of the inverse temperature transition. Figure 14 of PART 1 (Urry, 1987) demonstrated this with synthetic polypeptides of different hydrophobicity and oxidative processes demonstrated the resulting increase in temperature for the inverse temperature transition in Figures 11 and 12. The challenge for mechanochemical coupling of the first kind then becomes one of changing the hydrophilicity reversibly. In Figure 13A, a largely extended series of  $\beta$ -turns coil up into a regular  $\beta$ -spiral structure as the structural change of an inverse temperature transition where intramolecular hydrophobic side chain interactions are optimized on forming a spiral with interturn hydrophobic contacts. If the hydrophobicity can be decreased while setting at 37°C, for example, then the transition temperature would be raised and the spiral structure would unwind, turning off elastomeric force.

In mechanochemical coupling of the first kind, polypeptide elastomeric force develops on going from a higher entropy state to a lower entropy state, i.e., due to an inverse temperature transition. In mechanochemical coupling of the second kind, a change in chemical potential shifts the temperature for a regular transition for going from a less elastic, lower entropy state to an elastic, higher entropy state. The chemical process would lower the temperature of the transition to turn "on" the elastomeric force. In the example in Figure 13B, an inelastic  $\alpha$ -helix which is an extended state is converted to a highly elastic spiral state which then shortens to the extent allowed by the load. The result is an elastic contraction. Any chemical process which raises the free energy of the  $\alpha$ -helix and/or lowers the free energy of the spiral state would bring about a contraction.

What is here called mechanochemical coupling of the second kind is what has generally been meant by mechanochemical coupling as for example by free energy transduction in muscle contraction (Eisenberg and Hill, 1985). Mechanochemical coupling of the first kind becomes possible on having demonstrated that elastomeric force can develop as the result of an inverse temperature transition. This latter insight is uniquely derived from the elastin studies.

A. Elastic Molecular Machines and a Motive Force in Protein Mechanisms

1. Using Temperature Change to Turn "On" and "Off" Elastomeric Force

The lifting of a weight against gravitational force is the performance of work and a device capable of performing work may be called a machine. The cross-linked polypentapeptide of elastin is an elastic molecular machine. As shown in Figure 14A, when a strip of cross-linked polypentapeptide of elastin is attached to a weight at 20°C and the temperature is raised to 40°C, the weight is lifted (Urry, et al., 1986). This is a much greater shortening than occurs for example for latex rubber where the change is only 5% for the same temperature interval and weight. The reason for the performance of more work by the polypentapeptide is the inverse temperature transition that occurs between 20° and 40°C. The magnitude of the change that occurs without a load is seen in Figure 14B. The length change is the result of the wrapping up of the largely extended series of  $\beta$ -turns into a  $\beta$ -spiral structure as shown in Figure 13A. By analogy, this could be called thermomechanical transduction of the first kind.

Temperature change may also be used to turn on elastic force in the case of the structural changes considered in situations relevant to mechanochemical coupling of the second kind. A weight may be attached to the end of an  $\alpha$ -helix as shown in Figure 15A. If there were a graded instability of the helix with respect to an increase in temperature with the lower portion of the helix being less stable, then on raising the temperature, the lower part of the

helix undergoes transition first as shown in Figure 15B. A portion of  $\alpha$ -helix is converted to an extended elastic spiral structure which shortens lifting the weight. A further rise in temperature converts additional  $\alpha$ -helix to spiral and further lifts the weight. It is helpful to have the graded instability in order to facilitate reversibility in a condensed system. This could be called thermomechanical transduction of the second kind. If instead of a temperature change, the temperature of the transition could be lowered by a chemical process, then mechanochemical coupling of the second kind would be achieved.

## 2. Using a Change in pH to Turn "Off" and "On" Elastomeric Force

The principle of using pH change as the chemical process for mechanochemical coupling of the first kind can be shown by the effect of introducing an occasional Glu residue in position four of the polypentapeptide. When the ratio of Glu to Val in position four is 1:4, the temperature for the inverse temperature transition for the onset of the intermolecular component of the inverse temperature transition is raised from 25°C for the pure polypentapeptide to 37°C at pH 2 for the Glu containing polypentapeptide. The change of one residue in 25 from Val to Glu (COOH) increases hydrophilicity (polarity) sufficient to shift the transition by 12°C. On raising the pH to 6, the temperature for the onset of aggregation of the 20% Glu<sup>4</sup>-polypentapeptide is raised to 49°C. The change of one side chain in 25 residues from COOH to COO<sup>-</sup> again increases the temperature of the transition by 12°C. This is shown in Figure 16. A cross-linked matrix for demonstrating shifts in elastomeric force development has yet to be formed but it is expected that it will provide the first demonstration of mechanochemical coupling of the first kind. In order that the temperatures for the transitions be lower, the Ile<sup>1</sup>-PPP can be used. The principle is shown in Figure 17 in terms of thermoelasticity curves. Elastomeric force develops between 20° and 40°C. If the polypeptide elastomer is made more

polar for example by ionization of the carboxyl side chains of the included glutamic acid residues, then the transition is shifted some 20°C higher in temperature and comparable elastomeric force does not develop until 60°C. A change in the pH from 2 to 7 at 37°C would change from one curve to the other; and elastomeric force would be turned off by this change in chemical potential. Thus just as changing the temperature from 20° to 40°C raises the weight in Figure 14, lowering the pH from 7 to 2 at constant temperature is expected to turn on an elastomeric force and perform work, e.g. lift a weight. As this occurs due to the development of an entropic elastomeric force, it could appropriately be called entropic motive force (EMF).

It should be noted that almost four decades ago, Katchalsky (1951) demonstrated mechanochemical coupling with polyelectrolytes and considered the structural interconversions from rigid sphere at low ionization to statistical (random) coils at about 10% ionization to extended rigid rods at high ionization. This would be relevant to mechanochemical coupling of the second kind.

### 3. Possible Chemical Processes at Constant pH and Temperature

In general, for mechanochemical coupling to be of use to warm blooded animals, it would best occur at a constant temperature and pH. Transient local pH changes would seem to be of limited use; since proton diffusion is so much faster than the structural change, the pH change will have dissipated before the structural response can have occurred. In those special cases where a change of pH were maintained for sufficiently long times, of course, pH change would be a useful on/off switch.

#### a. Deamidation/Amidation as an On/Off Switch

If deamidation of glutamine and/or asparagine in a polypeptide chain could be achieved, then at pH 7 the result would be the production of an anionic carboxylate side chain. This is equivalent to the deprotonation of



the carboxylic acid side chain shown in Figure 16 and would on deamidation raise the temperature of the inverse transition of a 20% Gln<sup>4</sup>-PPP with the result of turning off elastomeric force (see Figure 17). Conversely, the amidation of a carboxylate side chain could be used to turn on elastomeric force. While glutamine transaminase is a well-known enzyme system, it acts on the free amino acid. A search of the literature for amidases turned up no enzymes that could function as an amidase for glutamine or asparagine while in a polypeptide chain. There is reported an amidase-like activity of calpain (Ca<sup>2+</sup>-dependent cysteine proteinase) I and II which hydrolyzes the carboxyl-terminal amide of substance P but leaves the glutamine residue of substance P intact (Hatanaka, et al., 1985). There is also a so-called  $\omega$ -amidase pathway for the degradation of glutamine which converts a carboxamide to a carboxylate but the action is on  $\alpha$ -ketoglutaramate to produce  $\alpha$ -ketoglutarate and ammonia (Calderón, et al., 1985).

b. Dephosphorylation/Phosphorylation as an On/Off Switch

The use of phosphorylation to activate a group such as a carboxyl for synthesis in a metabolic process such as in the glutamine transaminase catalyzed reaction is reasonably well understood. Just how the energy of adenosine triphosphate hydrolysis is used in muscle contraction, in cell motility, in modulation of enzyme processes and channels, in membrane transport, etc., is less well understood. The timely release of kinases for phosphorylation and phosphatases for dephosphorylation is central to mechanochemical coupling in cell motility. The mechanism that emerges from the study of the elastic polypentapeptide and its analogs is the turning on and off of elastomeric force by changing the temperature of a transition between two states, one of which can exert an elastomeric force. Just as ionization changes the hydrophobicity of the elastomeric sequential polypeptide and thereby changes the temperature of an inverse temperature transition, phosphorylation of a polypeptide

by Figure 17 could similarly be expected to change the temperature of an inverse temperature transition and to turn off an elastomeric force while dephosphorylation could turn it back on. This would be a case for mechanochemical coupling of the first kind (see above). As has been more commonly appreciated, it would also be possible for a change in phosphorylation to shift the equilibrium for a standard transition from a lower to a higher entropy state as the on/off switch in mechanochemical coupling of the second kind.

c. Changes in Calcium Ion Activity as an On/Off Switch

Calcium ion has the capacity to bind polypeptide and protein with binding constants as large as  $10^6/M$  or greater. This means that changing calcium ion activity could also be used as an on/off switch for shifting between two states, one elastomeric and the other relatively inelastic. The binding of calcium ion to troponin C, calmodulin, parvalbumin and related proteins is well-appreciated and in the former two cases are an important element in determining the state of muscle contraction in striated and smooth muscle. The introduction of a  $\Delta pCa$  as a mechanism for turning elastomeric force on and off in the present context of the possible implications from understandings developed on the sequential polypeptides of elastin and their analogs, could relate to consideration of the repeating sequences in myosin and possible modifications of the Harrington (1971, 1979) and the Huxley and Simmons (1971) models for the occurrence of elastic forces in muscle contraction (see below.)

B. Events Attending Parturition and their Reversal

The remarkable changes that facilitate birth and their reversal center on two anatomical sites: the pubic symphysis and the uterine cervix. In the days immediately preceding delivery, the cartilaginous pubic symphysis converts to an interpubic ligament which relaxes the size constraints on the pelvic girdle (birth canal) and the uterine cervix softens and can open wide to allow natural

birth to occur. Both development of interpubic ligament and cervical softening occur in response to the relaxation factor first recognized by Hisaw (1926) in the guinea pig. The relaxation factor has been identified as an insulin related hormone called relaxin (for reviews see Kemp and Niall, 1984; Weiss, 1984). Both in the pubic symphysis (Braddon, 1978) and the uterus (Judson, et al., 1980), a response to the administration of relaxin is the increase in concentration of cyclic adenosine monophosphate (cAMP) which activates a cAMP dependent kinase (Kemp and Niall, 1984). This enzyme phosphorylates serine and threonine residues in sequential proximity to arginine and lysine residues (Sparks and Brautigan, 1986). The phosphorylation of protein associated with a relaxation of tension immediately suggests mechanochemical coupling of the first kind discussed above which perhaps even more interestingly also provides the basis for reversal, i.e. for the active pulling of the structures back to their near predelivery state by dephosphorylation.

#### 1. Interpubic Ligament Formation and Reversal

In virgin mice, the pubic bones are separated by less than one millimeter; in the half-dozen days before parturition, the gap increases becoming bridged by a 5 - 6 mm long ligament at term; after delivery, "the gap rapidly closes" to less than 2 mm (Hall, 1947). This is illustrated in Figure 18. The dominant protein components of ligament are elastin and collagen, there being 80 - 90% elastin in ligamentum nuchae. As collagen is non-extensible, partial removal or detachment would seem necessary in order for elongation to occur. Elastin present could simply unwind on phosphorylation if an appropriate extracellular kinase activity were present (Kübler, et al., 1983). It has been seen with the polypentapeptide of elastin that the relaxing of force that occurs on the low temperature side of the inverse temperature transition leads to an extension by a factor approaching 2.5 in the absence of an extending force (a

load) (see Figure 14 above and Figure 13 of PART 1, Urry, 1987). At a constant temperature, the relaxation would occur as phosphorylation (increased polarity) raised the temperature of the transition as shown in Figure 17. If there were no extracellular kinase, tropoelastin could be phosphorylated intracellularly, for example by the cAMP dependent kinase, and then elastin fibers would be formed extracellularly in the relaxed, extended state. Interestingly, as shown in Figure 1 for bovine ligamentum nuchae elastin, there are serines and threonines in sequential proximity to arginines and lysines. It might be inquired as to why all ligaments, and elastin containing tissues, would not be equally relaxed in late pregnancy. The answer could be the de novo synthesis of elastin by cells at the targeted anatomical sites and/or it could involve the interesting splicing-out of sequences between cross-links that has been observed by Indik, et al., (1987) and/or it could involve the localized release of extracellular kinases (Kübler, et al., 1983). Perhaps more significant than the relaxing effect would be the capacity to turn on the elastomeric force, to shorten the ligament and draw the pubic bones back toward their pre-relaxed dimensions as depicted in Figure 18. Extracellular dephosphorylation following delivery would provide the mechanism for decreasing chain polarity and for turning on elastomeric force by the winding up of the  $\beta$ -spiral structure as shown in Figure 13A, thereby drawing the pubic bones back to their near predelivery state.

## 2. Cervical Ripening

In the uterine cervix, there are elastin fibers that could also undergo relaxation by phosphorylation as proposed for the interpubic ligament. If there are titin-like proteins in smooth muscle, however, as in striated and cardiac muscle (Wang, 1985), then phosphorylation of this or a similar elastic protein would be another means of relaxing the cervical closure and dephosphory-

lation could result in reformation of the near nulliparous uterine cervix. This would be relevant to the uterus in general. As noted above in part IIB, titin is isolated with 2 to 3 moles of phosphate per mole of protein (Wang, 1985). Again, it is not so much the relaxation and softening that is remarkable as it is the reversal of the effects and the development of the forces necessary to return to the near normal cervical opening. Should such a capacity exist in smooth muscle cells to modulate tension exhibited by a supramicron length elastic protein, then the role of such a protein in some forms of essential hypertension would become of interest. In smooth muscle, it might be noted in consideration of possible relevance of the elastin studies in relation to the effects of phosphorylation/dephosphorylation as an on/off switch for structural change that there occurs phosphorylation by myosin light chain kinase (Dabrowska, et al., 1977) and reversal by myosin light chain phosphatase (Aksoy, et al., 1976) which accompanies smooth muscle contraction and that proteolytically modified myosin light chain kinase can activate actin dependent myosin ATPase resulting in contraction without calcium ion or calmodulin (Walsh, et al., 1983).

### C. Muscle Contraction

Muscle contraction, can be characterized as the turning on and off of elastic forces (Hibberd and Trentham, 1986). Accordingly, the insights provided by the studies on elastin peptides and related analogs, the new perspective on the source of entropic elastomeric force that result, and the concepts that emerge for turning elastic forces on and off hopefully justify a few considerations from one not expert in the area of muscle contraction. In keeping with phosphorylation and dephosphorylation as a means of altering chain polarity and of reversibly shifting the temperature for a structural transition between highly elastic and less elastic states of different end-to-end chain lengths, in

relation to the interactions with ATP, one means whereby the elastin results could be relevant would be to the proposed structural changes involving the head or S-1 fragment of myosin. In this case, length changes could be considered which might relax the requirement for large angle changes in cross-bridge rotation. One scenario could be ATP dependent relaxation allowing the cross-bridge to extend and attach to the actin filament followed by release of phosphates and elastic shortening for the powerstroke.

The foregoing understandings of elasticity were developed on polypeptides comprised of repeating sequences and the new class of conformations (spirals) that result are intermediate in entropy between  $\alpha$ -helices,  $\beta$ -sheets, for example on the one hand and random chain networks or random coils on the other. The obvious repeating sequences, heptamers grouped as 28mers (McLachlan, 1984), in the myosin rod segment suggest consideration of elastic spiral structures in muscle contraction. Studies on the  $\beta$ -spirals of the polypentapeptide, polytetrapeptide, polyhexapeptides and polynona peptides of elastin indicate spiral structures of the order of 15 (Venkatachalam and Urry, 1981; Urry, et al., 1981), 16 (Khaled, et al., 1985), 12 (Urry and Long, 1976) and 18 (Chang, et al., unpublished data) residues per turn, respectively, with values in the range of 0.5Å/residue for translation along the spiral axis in the relaxed state. A spiral structure with some 14 residues/turn would seem possible for the repeat sequences of the myosin rod. With the concentration of positive charge in every fourth heptamer (McLachlan, 1984), a 14 residue/turn spiral would have the positive charge in alternate turns. Such a spiral structure could provide for an elastic collapse of length on going from  $\alpha$ -helix to spiral as indicated in Figure 13 and 15. A structural transition of this type could be relevant to the proposed mechanisms of Huxley and Simmons (1971) and Harrington (Harrington, 1971, 1979; Tsong, et al., 1979, 1983). Utilizing a chemical pro-

cess to lower the temperature of the transition in an  $\alpha$ -helix to spiral transition would seem to overcome the Skolnick (1987) criticism of the Harrington model of an  $\alpha$ -helix to random coil transition in the S-2 segment of the myosin rod. What is considered here would be a modification of the Harrington model where a non-random spiral structure of the order of 0.5Å/residue replaced the random coil and where a chemical process such as a change in the activity of calcium ion or change of polarity by phosphates would lower the temperature of the transition and trigger contraction. In the above noted case of smooth muscle, myosin light chain phosphorylation/dephosphorylation (Dabrowski, et al., 1977; Aksoy, et al., 1976; Walsh, et al., 1983) could provide a switching mechanism. The added negative charge of the phosphate could, by electrostatic repulsion, destabilize the  $\alpha$ -helix much as the  $\alpha$ -helix poly-L-glutamate is destabilized at a greater than 0.3 degree of ionization (see Figure 35 of Urry, 1968). With the demonstrated irreversibility of thermal denaturation in the condensed phases of the polypentapeptide of elastin (see PART 1) and of elastin itself (see Figure 4) where random coils presumably do develop, the conversion from  $\alpha$ -helix to spiral could be expected to occur with greater reversibility. Also with the low elastic modulus implied on complete thermal randomization of chains in elastin (see Figure 4), random chains do not appear to be the source of sufficient elastic moduli whereas spiral-like structures which derived their entropic elastomeric force from internal chain dynamics could provide the elastic modulus required in muscle contraction.

Consideration of the charge distribution in the rod segment shows the greatest excess of negative side chains over positive side chains to occur in the range of the S-2 segment, residues 300' to 520' in the McLachlan (1984) numbering, and the excess negative charge persists but decreases as one proceeds in both directions toward the amino and carboxyl ends. The charge distribution

suggests that this segment be a focal point for the collapse of  $\alpha$ -helix to spiral, and as the change in chemical potential progressed, the collapse could spread in an orderly fashion in both directions from a focal point. Such a double zipper mechanism (a term made perhaps even more appropriate by the dimeric  $\alpha$ -helical structure of the myosin rod) could facilitate reversibility and could possibly provide explanation for the long stroke (sliding) distance of greater than 600A for a single ATP cycle reported by Yanagida, et al., (1985). The very interesting stretch activation exhibited by insect flight muscle and possibly mammalian heart muscle (Pringle, 1978) could be a mechanical stretching which converted spiral back to  $\alpha$ -helix even under chemical conditions which in the absence of the mechanical stretch would favor elastic spiral over  $\alpha$ -helix. Stretch activation could also be relevant to the possible length changes considered above in the S-1 fragment. In terms of the two kinds of mechanochemical coupling noted above, that of the putative involvement of the S-1 segment could possibly be mechanochemical coupling of the first kind whereas that of the S-2 segment and the rod in general could be mechanochemical coupling of the second kind.

#### D. Cell Motility

As with the other protein systems and processes considered above, in this section on cell motility, molecular mechanisms are put forward that interject the concepts of elasticity and of turning on and off elastomeric force which were derived from studies on the elastin system; the purpose (as above) is to stimulate inclusion of these perspectives in consideration of, in this case, cell migration by those who are expert in this area. The points that are drawn from the elastin system are the analogy between coacervation and a sol  $\rightarrow$  gel transformation and the effects of changing polypeptide chain polarity in a way that relaxes elastic constraints. Another but not obligatory point that can be



introduced is the demonstration in the fibronectin-fibroblast system that cell attachment and chemotaxis can utilize the same peptide sequence (Long, et al., 1987).

Perhaps an interesting starting point would be one in which the only directionality present would be due to a concentration gradient of chemoattractant (CA) as depicted in Figure 19. Consider a cell of essentially circular shape attached to its appropriate substrate. Within the cell is a cytoskeletal network in which microfilaments span from cell membrane to cell membrane, crisscrossing the cell as an essentially isotropic network. Importantly, these microfilaments exert an elastic constraint on the cell membrane. The chemoattractants on the high concentration side of the cell bind to their receptors with the following sequelae. With the possibility of a commonality of cell attachment peptide sequence and chemotactic peptide sequence, the chemoattractant competitively releases cell attachment to the substrate and binds to the cell receptor with the result of localized cAMP release within the cell. The released cAMP locally activates cAMP dependent kinases which phosphorylate nearby elastic microfilaments, for example one end of a titin-like molecule. Phosphorylation raises the temperature of the inverse temperature transition and causes the elastic microfilament to unwind and to turn off the local elastomeric constraints on the membrane. The unwinding of the microfilament has elements equivalent to the reversal of coacervation, that is, a gel  $\rightarrow$  sol transition which causes an osmotic flow of water to the site much as the cross-linked polypentapeptide of elastin swells with a factor of ten increase in volume on lowering the temperature below the onset of the inverse temperature transition (see Figure 14B). Raising the temperature of the inverse temperature transition by phosphorylation at a fixed temperature is equivalent to lowering the temperature to below the onset of the inverse temperature transition. Phosphory-

lation would be the equivalent in Figure 17 of going at 37°C from the solid curve where the elastomeric force is turned on to the dashed curve where elastomeric force is turned off.

With the simultaneous local relaxation of elastic constraint on the membrane and the local swelling of the gel  $\rightarrow$  sol transition, the cell membrane protrudes by pseudopod formation in the direction of increasing concentration. By these processes, an isotropic cell becomes anisotropic and achieves movement in the direction of the concentration gradient. Reattachment to the substrate, dephosphorylation of the local microfilaments, possibly engaging the acto-myosin motive system with organization of stress fibers to draw up the rear of the cell could all presumably follow appropriately. It may be noted that very recently cell migration in a concentration gradient has been shown to occur apparently without functional myosin (Solomon, 1987; Knecht and Loomis, 1987; DeLozanne and Spudich, 1987). This directs attention toward elastic proteins and forces outside of the acto-myosin system. The foregoing is but one of a number of possible scenarios that could utilize what has been learned from studies of the nature of polypeptide elasticity.

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Figure Legends:

Figure 1 Primary structure of bovine ligamentum nuchae elastin. This is the sequence data reported by Indik, et al., 1987, arranged according to sequences between cross-link forming lysines and labelled according to Sandberg, et al. (1985) in analogy to porcine aortic elastin. The recognized desmosine and isodesmosine forming sequences are bold faced and are KAAK and KAAAK generally associated with strings of alanines, A. At the heart of the primary structure is the longest sequence, W4; it contains the polypentapeptide of elastin which was extensively characterized in PART 1 (Urry, 1987). The next longest sequence, W6, contains a repeating hexapeptide followed in length by RP-71 (W14) which contains a repeating nonapeptide. Also notable are serines, S, threonines, T, and tyrosines, Y, in sequential proximity to charged lysine, K, or arginine, R, residues. These are potential phosphorylation sites.

Figure 2 Transmission electron micrographs of negatively stained incipient coacervates of tropoelastin, A, and of  $\alpha$ -elastin, B, with optical diffraction patterns included. The primary diffraction spots are near 5 nm. It seems apparent on raising the temperature of aqueous solutions of tropoelastin and of  $\alpha$ -elastin that these molecules molecularly dispersed in solution below 20°C can self-assemble into filamentous arrays on raising the temperature to 37°C. This demonstrates the intermolecular component of an inverse temperature transition. Micrographs reproduced with permission from Cox, et al., 1973, 1974, and diffraction patterns from Volpin, et al., 1976.

Figure 3

Thermoelasticity study of bovine ligamentum nuchae elastin at 60% extension measured at 40°C. In the upper curve, the temperature was raised from 20°C at a rate of 4 hours per data point to 40°C then at a rate of 30 min. per data point. The temperature was then reduced to 40°C and the temperature raised at a rate of 4 hours per data point. When the temperature is raised at the faster rate above 40°C, a near zero slope is obtained. The curve is not reproduced on lowering the temperature and a slower rise in temperature shows a marked loss of elastomeric force above 55°C. As will be confirmed in Figure 4, this irreversible change is due to thermal denaturation.

Figure 4

Series of stress/strain curves for ligamentum nuchae elastin determined at 40°C to an extension of 60%. Between subsequent curves the sample was returned to zero extension and heated at 80°C for approximately 24 hours. Heating at 80°C results in a progressive loss of elastic modulus. A plot of  $\ln(\text{elastic modulus})$  versus time at 80°C is given as an insert from which a half-life of about 10 days is obtained. This data and that of Figure 3 demonstrate a slow thermal denaturation of ligamentum nuchae elastin. Thermal randomization of chains causes loss of elastic modulus (Urry, Haynes and Harris, unpublished results). This is difficult to reconcile with the perspective that the source of elastomeric force is due to random chain networks and argues, where significant elastic forces are observed in protein mechanisms, that non-random coil structures require consideration.

Figure 5

Temperature dependence of the dielectric permittivity (real part) in the 1 MHz to 1000 MHz frequency range of the  $\alpha$ -elastin coacervate. On raising the temperature, a localized relaxation develops near 20 MHz. The temperature dependence of the correlation time

in the insert indicates an activation energy for polypeptide backbone motion of approximately 1.5 kcal/mole, a value also found from NMR studies of the polypentapeptide of elastin. This backbone librational process is labelled the  $\lambda$ -relaxation. Reproduced with permission from Urry, et al., 1985.

Figure 6

Electron micrographs showing titin to form long filamentous structures. The calibration bars are: 1  $\mu\text{m}$ , above, and 0.2  $\mu\text{m}$ , below. The lengths of the titin molecules are of the order of 1  $\mu\text{m}$  with diameters of 4 to 5 nm. Circled below is a myosin dimer showing the two heads (cross-bridges). Reproduced with permission from Wang, et al., 1984.

Figure 7

Above: Two-filament model comprised of thick filaments of myosin centrally aligned in the sarcomere and thin filaments originating at the Z-line and interdigitating with the thick filaments. Below: Three-filament model where the third elastic filament runs from the Z-line into the thick filament. The third filament is shown to be continuous when the muscle has been stretched beyond overlap of thick and thin filaments. Reproduced with permission from Magid, et al., 1984. From the work of Wang and colleagues, the third filament (titin) is reported to span from the Z-line to the M-line at the midpoint of the thick filament.

Figure 8

Relative permittivity (real part) of muscle tissue. The  $\beta_1$ -relaxation occurs in the frequency range that has been identified in the polypentapeptide of elastin (see Figure 12 of PART 1, Urry, 1987) and in elastin (see Figure 5 above) as due to peptide backbone librational motions where it was labelled the  $\lambda$ -relaxation. Reproduced with permission from Grant, et al., 1980, using data from Schwan, 1974.

Figure 9

Representations demonstrating the capability of an enzyme to produce oriented stresses (elastic forces) which are to be considered in achieving strain in a scissile bond of a substrate. In the "BAD" example, the mobility is more random in nature, and well-oriented, catalytically useful stresses do not result. In the "GOOD" example, the motions occur in such a manner as to produce oriented elastic forces that can produce strain in a substrate bond. Reproduced with permission from Gavish, 1986. The "BAD" result would be exemplified by elastic forces in a random coil or random network of chains whereas the "GOOD" result can occur when elastic forces derive from changes in internal chain dynamics in a non-random structure as in the librational entropy mechanism of elasticity.

Figure 10

Schematic representation of an elastic chain segment resulting in bond strain during enzyme catalysis.

- A. The enzyme active site before interaction with substrate with a relaxed elastic chain segment shown as a spiral structure.
- B. On binding of the substrate in an induced fit process, the elastic segment becomes stretched which effects a damping of motion in the polypeptide backbone and in the side chain that interacts electrostatically with atom B. This results in an oriented stress which produces a strain in the A-B scissile bond.
- C. An enzyme intermediate with B covalently attached to the side chain that is contiguous with the polypeptide chain segment where binding of substrate caused damping of librational motions.
- D. The regenerated enzyme with relaxed elastic chain segment and with products of the catalysis.

While the elastic polypeptide chain segment is represented as a spiral, it could involve as few as two or three residues. Substrate binding causes an effective extension of a chain segment and damping of librational motions within it. This results in the development of an entropic elastomeric force that can produce a strain in the scissile bond thereby facilitating bond cleavage.

Figure 11

Temperature profiles for the coacervation (aggregation) of the polypentapeptide of elastin in which the Pro residue has been partially or totally replaced by hydroxyproline (Hyp). Curve a is 0% Hyp, i.e.,  $(\text{Val}^1\text{-Pro}^2\text{-Gly}^3\text{-Val}^4\text{-Gly}^5)_n$ ; curve c is when on the average one in 10 pentamers contains a Hyp, that is, one in 50 residues has been altered by addition of a hydroxyl moiety; aggregation is shown to be delayed on raising the temperature by 7°C. Curve d is 100% Hyp, i.e.,  $(\text{Val}^1\text{-Hyp}^2\text{-Gly}^3\text{-Val}^4\text{-Gly}^5)_n$  and onset of aggregation requires an increase in temperature by about 35°C. Increase in the hydrophilicity (i.e., a decrease in hydrophobicity) markedly raises the temperature for the onset of the inverse temperature transition.

Figure 12

Effect of a xanthine oxidase superoxide generating system on the elastic properties of ligamentum nuchae elastin. The experiment was followed for twelve hours at 37°C with the superoxide generating system being replenished every two hours. Oxidation of ligamentum nuchae elastin causes a systematic loss of elastic modulus and an increase in the percent elongation required before the elastomer begins to resist deformation. A plot of the  $\ln(\text{elastic modulus})$  versus time shown as the insert gives a half-life of about 12 hours whereas as seen in Figure 4 when heating at 80°C in the absence of the superoxide generating system, the half-life was as many days (Urry, Haynes, Long and Freeman, unpublished

data). The prediction from the previous studies that an increase in hydrophilicity at 37° would cause an unwinding of the elastin structure with loss of elastomeric force for a given extension appears to be borne out by the experimental result. Such oxidative processes in the lung would lead to loss of elastic recoil as found in pulmonary emphysema.

Figure 13

#### Structural Transitions for Mechanochemical Coupling

- A. Mechanochemical coupling of the first kind where a relatively less-ordered and less elastic state undergoes an inverse temperature transition to a more-ordered, more elastic state, and a reversible chemical process which changes the hydrophobicity of the polypeptide chain shifts the temperature of the inverse temperature transition. In the specific example, a relatively disordered, largely extended series of  $\beta$ -turns winds up into an elastic  $\beta$ -spiral structure, defined as a helical arrangement of  $\beta$ -turns, in which the intramolecular hydrophobic interactions are optimized. For a given polypeptide chain hydrophobicity, as for example for the polypeptide of elastin, this inverse temperature transition occurs on raising the temperature from 20° to 40°C. If at 37°C the polypeptide chain were made less hydrophobic (more hydrophilic) for example by a phosphorylation, then the transition temperature would be raised and the structure would unwind with loss of capacity to exert an elastomeric force. Phosphorylation would then have turned off elastomeric force.
- B. Mechanochemical coupling of the second kind where a relatively more-ordered, less elastic state undergoes a standard transition on raising the temperature to a less-ordered, more elastic state and where a chemical process can change the

example, a relatively inelastic  $\alpha$ -helix converts to a more elastic spiral structure which derives its elastic force from the increase in internal chain dynamics within the regular structure. Any chemical process which increased the free energy of the  $\alpha$ -helix or decreased the free energy of the spiral state would lower the temperature of the transition and could turn on an elastomeric force.

Figure 14

Sketch showing the capacity of the  $\gamma$ -irradiation cross-linked polypentapeptide of elastin to function as an elastic molecular machine.

- A. To a strip of cross-linked polypentapeptide at 20°C is loaded a weight. On raising the temperature from 20° to 40°C, the weight is lifted as the elastomer shortens to 70% of its original length (Urry, et al., 1986). Latex rubber also shortens on raising the temperature over this interval but the change is only about 5%. The reason for the greater capacity of the cross-linked polypentapeptide to lift a weight is due to the occurrence of an inverse temperature transition wherein the polypentapeptide chain wraps up into a  $\beta$ -spiral structure as shown in Figure 13A.
- B. The contraction, decrease in dimensions, of the cross-linked polypentapeptide of elastin without load on raising the temperature from 20° to 40°C. Whereas latex rubber expands on raising the temperature in the absence of a load, the cross-linked polypentapeptide of elastin contracts to about 40% of its original length (see Figure 13 of PART 1, Urry, 1987). This simple experiment clearly shows the remarkable difference between the elasticity of polypeptide in water and

that of a classical rubber. The chemical modulation of this inverse temperature transition provides for a new motive force in protein mechanisms.

Figure 15

Elastic contraction due to a temperature elicited structural conversion from an  $\alpha$ -helix to a spiral structure. This is the result of a standard transition from a more-ordered to a less-ordered state on raising the temperature. An elastic spiral structure is shown rather than a random coil structure because thermal randomization of the polypentapeptide of elastin (see Figure 16 of PART 1, Urry, 1987) and of elastin (see Figure 4 above) causes a loss of elastic force rather than an increase in elastic force. As drawn, the lower end of the  $\alpha$ -helix is more thermally labile and the thermal instability decreases as one progresses up the helix such that the structural transition occurs in a regular manner as the temperature is raised. This graded thermal instability would facilitate reversibility. Chemical modulation of the temperature of the transition would be mechanochemical coupling of the second kind.

Figure 16

Temperature profiles of coacervation, i.e., of the intermolecular component of the inverse temperature transition; for the polypentapeptide of elastin,  $(\text{Val}^1\text{-Pro}^2\text{-Gly}^3\text{-Val}^4\text{-Gly}^5)_n$ , in curve a; for 20%  $\text{Glu}^4$ -polypentapeptide at pH 2 where the carboxyl group is unionized ( $\text{COOH}$ ) in curve b; and for 20%  $\text{Glu}^4$ -polypentapeptide at pH 6 where the carboxyl group is ionized ( $\text{COO}^-$ ). The effect of decreasing the hydrophobicity (increasing the hydrophilicity) by introducing one glutamic acid residue in 25 residues raises the temperature for the onset of the inverse temperature by  $12^\circ\text{C}$ . Conversion of one residue in 25 from a  $\text{COOH}$  side chain to a



COO<sup>-</sup> side chain further raises the temperature for the onset of the inverse temperature transition by another 12°C. As this inverse temperature transition has been shown to turn on and off elastomeric force in the cross-linked polypentapeptide, this result raises the possibility of turning on and off elastomeric force at 50°C by changing the pH. This would be mechanochemical coupling of the first kind.

Figure 17

Curves demonstrating mechanochemical coupling of the first kind. Solid curve: Thermoelasticity curve of 20 MRAD  $\gamma$ -irradiation cross-linked polypentapeptide of elastin where on raising the temperature from 20° to 40°C there is a dramatic development of elastomeric force,  $f$ , plotted as  $\ln[f/T(^{\circ}\text{K})]$  versus temperature ( $^{\circ}\text{C}$ ). This development of elastomeric force is due to an inverse temperature transition. On making the polypeptide less hydrophobic which can also be stated as more hydrophilic or more polar, the temperature of the inverse temperature transition for the development of elastomeric force is increased to give the dashed curve. This effect on changing chain polarity of shifting the inverse temperature transition is shown above in Figures 11 and 16. When the change in chain polarity can be achieved reversibly, then elastomeric force can be turned on and off by a chemical process. Examples of chemical means could be ionization/deionization as in Figure 16 or phosphorylation/dephosphorylation. This effect of phosphorylation could be operational in a fibrous structural protein or within a globular protein to modulate function by turning on and off elastic forces.

Figure 18

Schematic representation of changes in the pubic symphysis that attend parturition following the descriptions of Hall (1947) due to studies on mice. In virgin mice, the connective tissue con-

necting the pubic bones, the pubic symphysis, is a dense connective tissue filling a gap of less than 1 mm. In the few days preceding delivery, the gap between the pubic bones has become an interpubic ligament 5 to 6 mm in length. After delivery, the gap closes to about 2 mm with the regeneration of the pubic symphysis. Based on the perspectives gained from the elastin studies, it is proposed that the elastic forces required to draw the pubic bones back together result from dephosphorylations which would result in the development of elastic spiral structures and the turning "on" of elastic forces. This would be an application of mechanochemical coupling of the first kind.

Figure 19

Schematic representation of possible cell migration by pseudopod formation in response to concentration gradient of chemoattractant, CA. At left, the cell membrane is elastically constrained by elastic filaments that criss-cross the cell. They could equally well span, for example, from the nuclear membrane to the plasma membrane. On the side of the cell where the concentration of chemoattractant is higher, binding to cell receptors would cause local intracellular release of cyclic adenosine monophosphate (cAMP) which locally activates cAMP dependent kinases. At the right, the kinases have phosphorylated locally the elastic filaments increasing the hydrophilicity of the polypeptide chains and causing the elastic filaments to unwind and to release the elastic constraint on the membrane. This relaxation of elastic constraint and the natural water uptake that attends the reversal of an inverse temperature transition results in pseudopod formation initiating cell migration in the direction of the increasing concentration of chemoattractant.

GGLGPGVKPAK

W13 PGVGLVPGLGAGLGALPGAFPGALVPGGPAGAAAAAYKAAAK

WBB AGAAGLGVGCGTGGVGGLGVSTGAVVPQLGAGVGAGVKPKK

W11B,C VPGVGLPGVYRPGGVLPGAGARFPGLGVLPGVPTGAGVKPK

WIO APGGGGAFA GIPGVGPF GGQGP GVP L GYP I KAP \*

W14b L P A G Y G L P Y K T G K

W110 L P Y G F G P G G V A G S A G K A G Y P T G T G V C P Q A A A A A A K A A A K

W7 LGAGGAGVLPGVGGAGIPGAPGAI PGIGGI AGVGAPDAAAAAAAKAAK

[illegible]

W86 F G A R G C V G I G G I P T F G V G P G G F P G I G D A A A A Q A A A A A K A A K

W3 1GAGGVCALGGLVPGAPGAI PGVPGVG GVP GVG I PAAAAAKAAAK

W6 AAQFGLGPGVGVAPG VGVPPGVGVAPGIGLGPGGVIGAGVPA AAAKSAAKAAAK

RP-71  
(M14)  
AOFRAAAGLPAGVPGLGVGVPGLGVGAGVPGFGAVPGTLAAAKAAK

#5 FCGGCGALGGVGDLCGAGIPCGVAGVCPAAAAAAXAAAX

W9 AA0FGLGGVGLGVGGLCAVPGA VGLGGVSPA AAKAAK

From FGAACLGGLGAGQPFPIGGVAARPGLGLSPIFGGAGGLGVGGKPK  
43

21  
21  
PFGGALGLGF PGGACL GKSSCGRRKX

FIGURE 1

A



B



FIGURE 2

Thermoelasticity Study  
ligamentum nuchae, 60 % extension

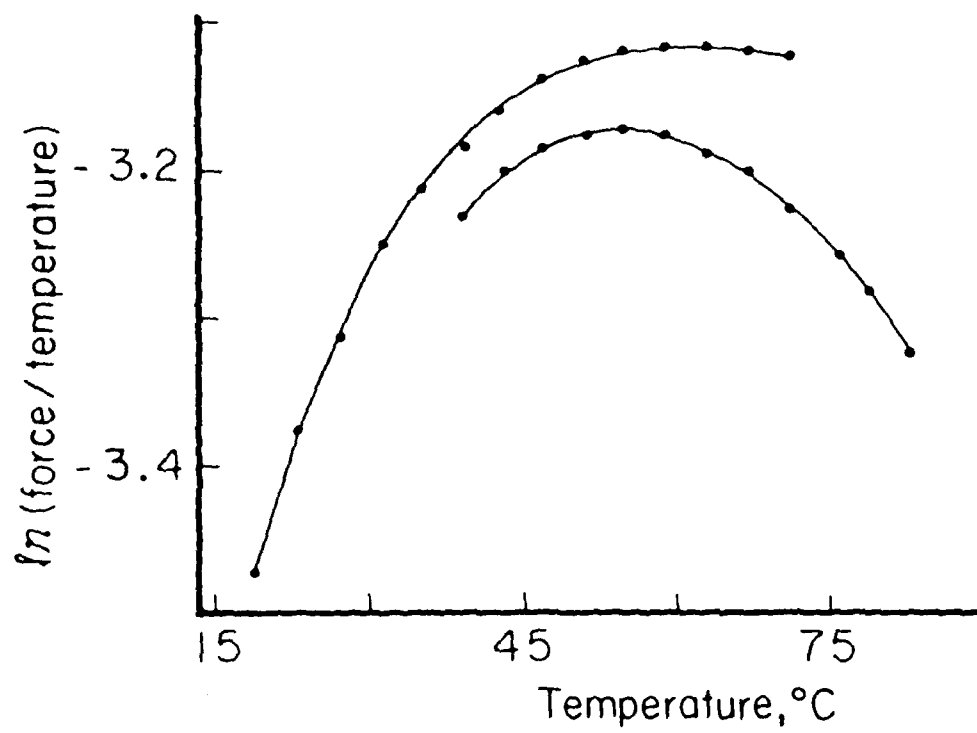


FIGURE 3

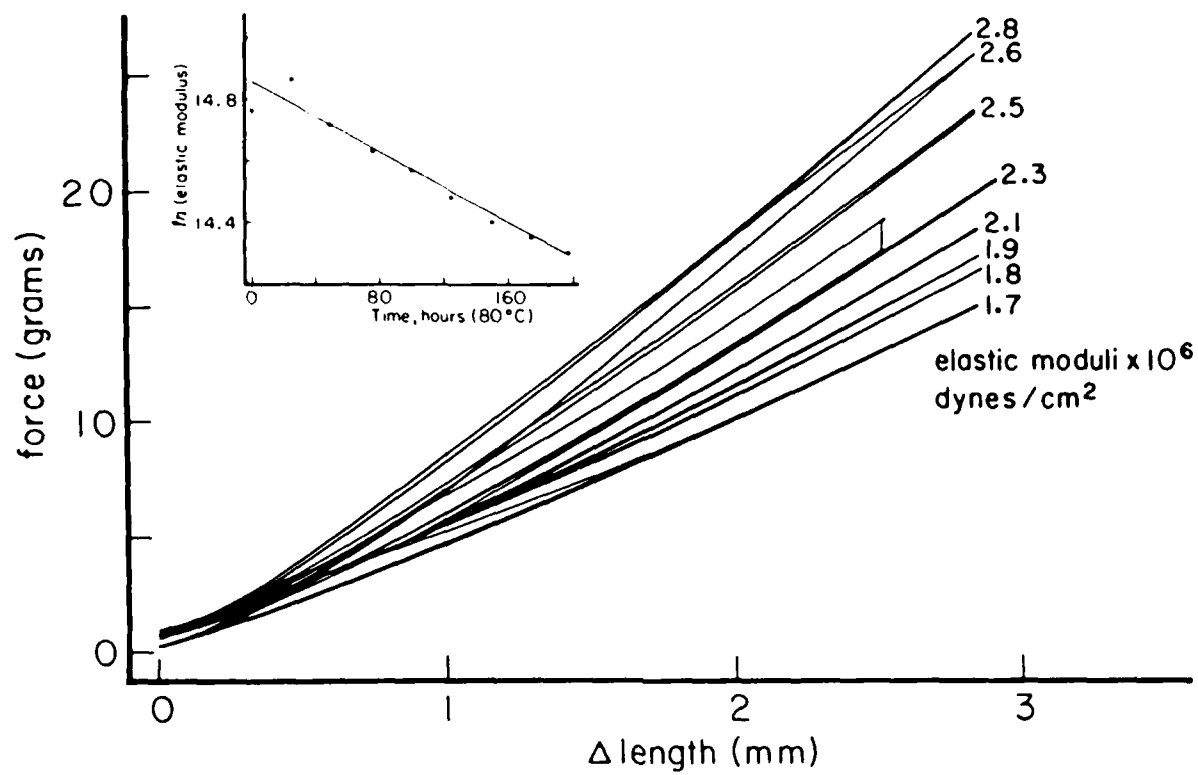


FIGURE 4

# TEMPERATURE DEPENDENCE DIELECTRIC RELAXATION SPECTRUM OF $\alpha$ -ELASTIN COACERVATE

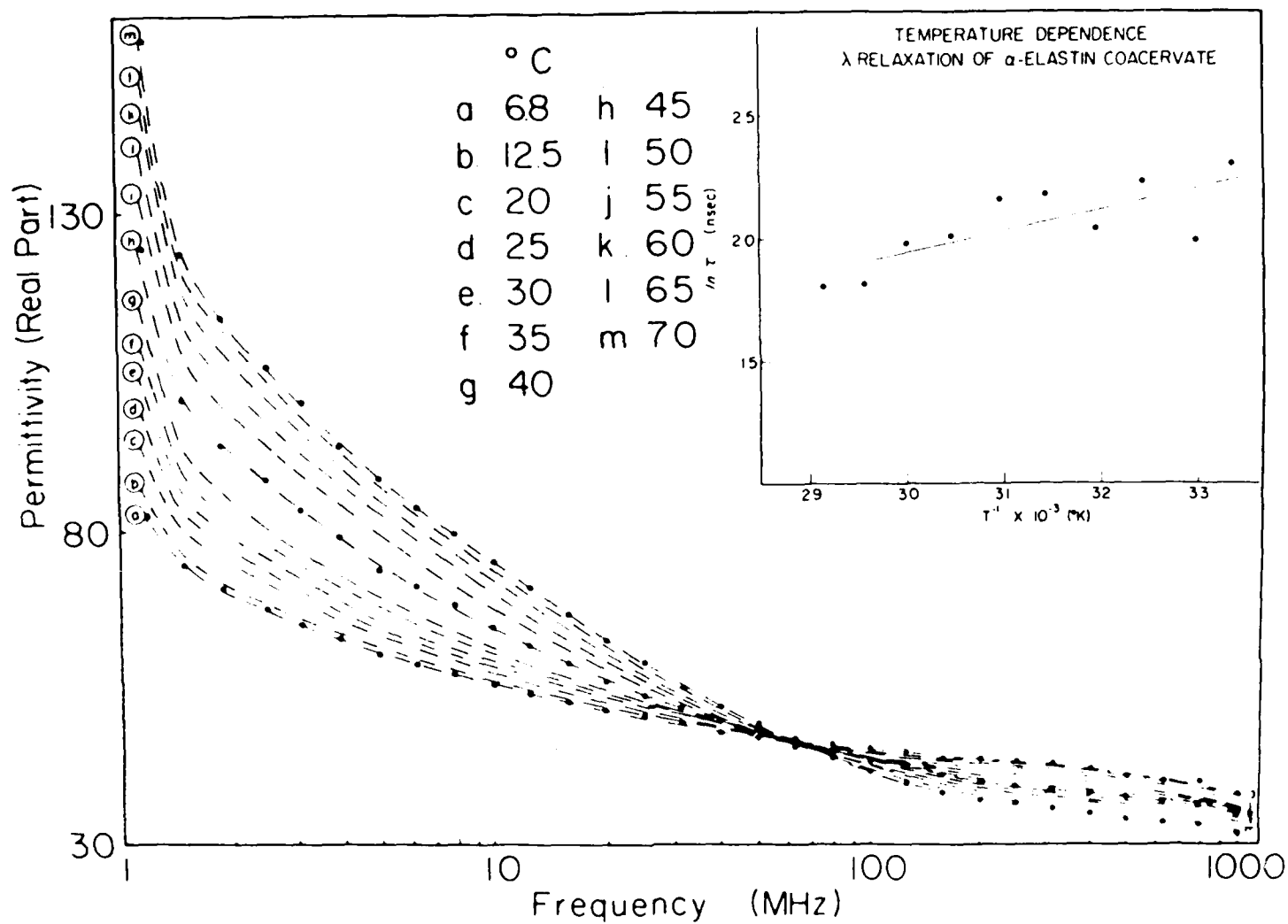


FIGURE 5

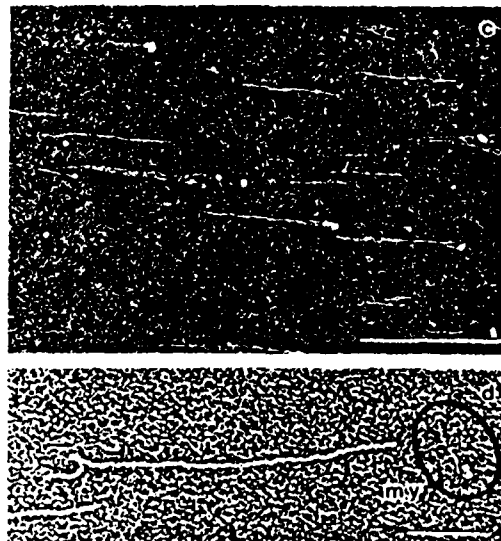
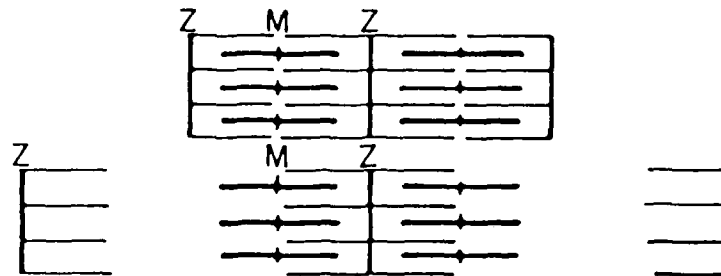


FIGURE 6



## Two-Filament Model



## Three-Filament Model

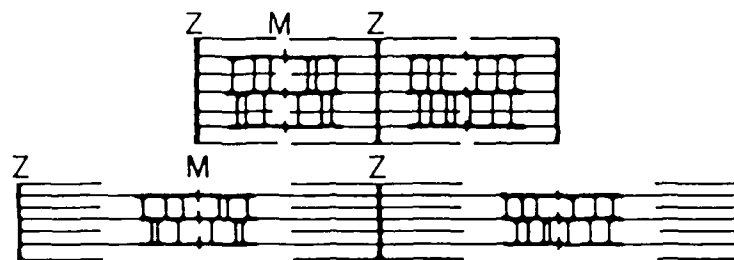


FIGURE 7

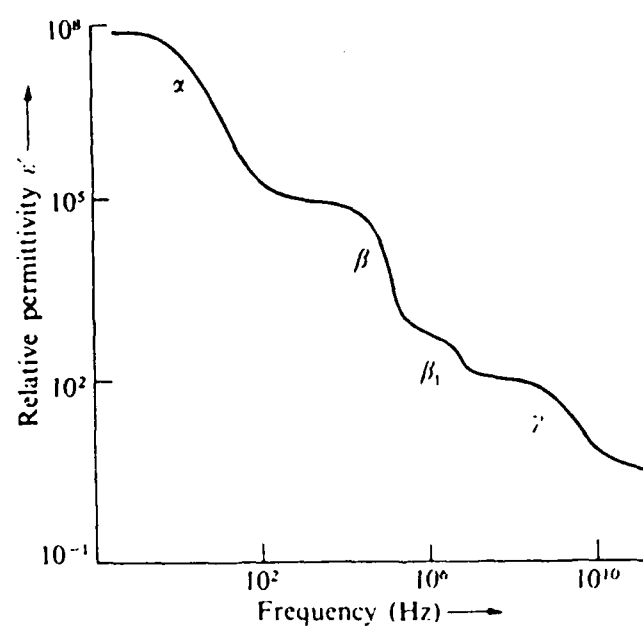


FIGURE 8

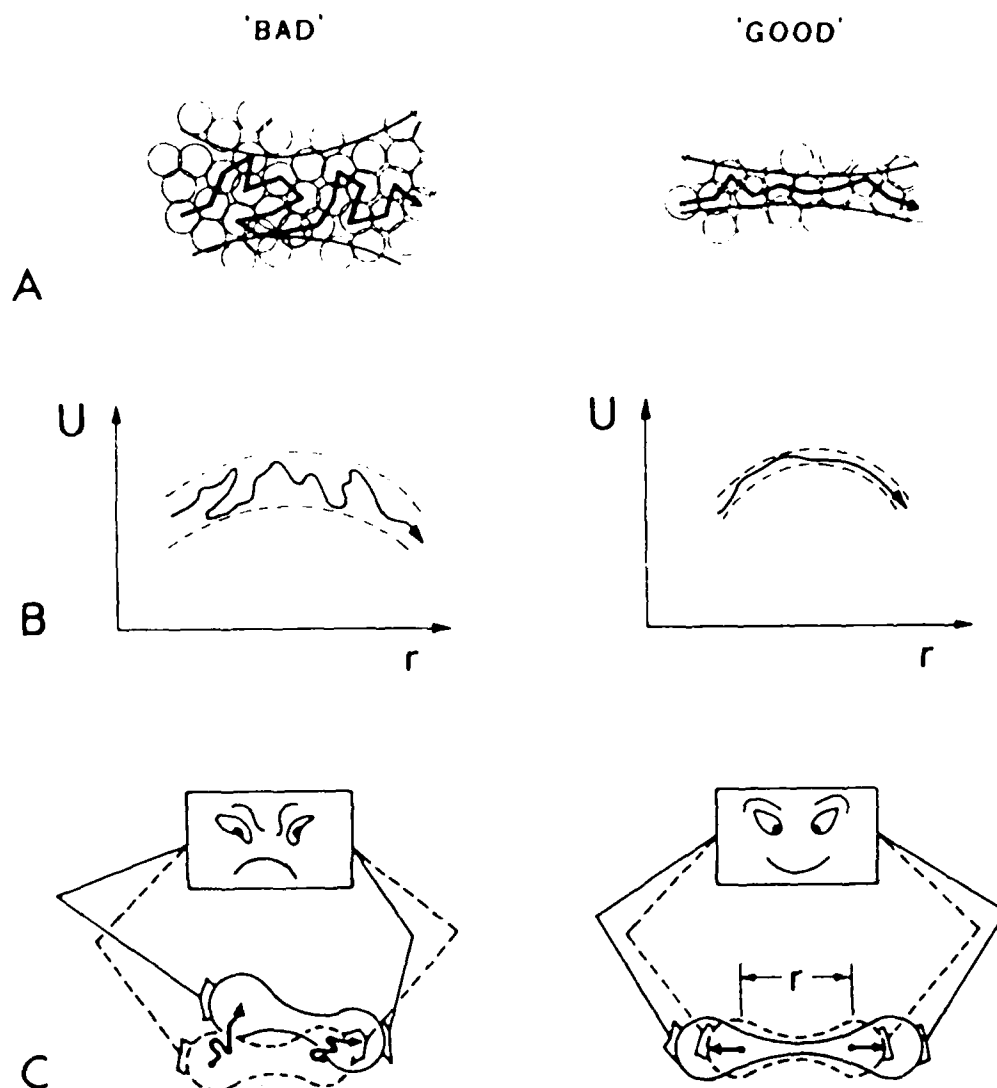
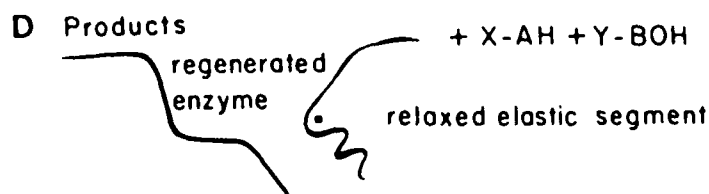
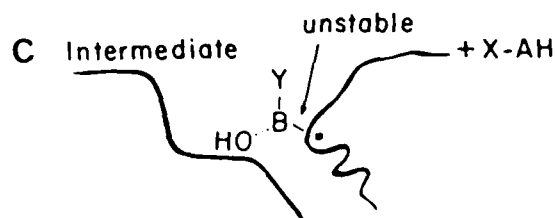
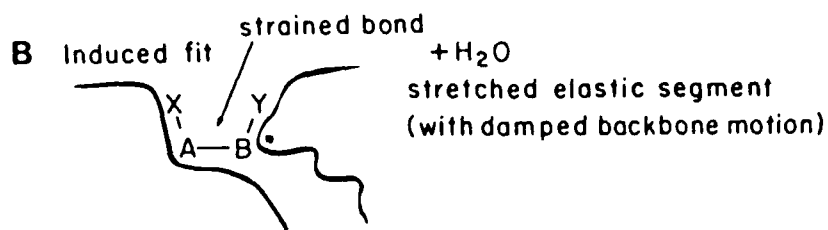
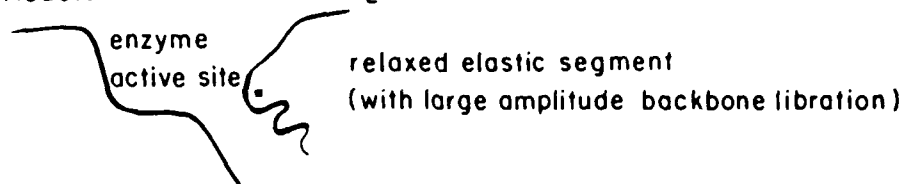


FIGURE 9

# Schematic Representation of Elastic Segment Contributing Bond Strain During Enzyme Catalyses

A Reactants  $X-A-B-Y + H_2O$



\*Elastic segment could involve as few as two or three residues.  
Binding results in effective extension and damping of librational motions.

FIGURE 10

Temperature Profile of Coacervation (1mg/ml)  
 Effect of Prolyl Hydroxylation in  $\text{HCO}-(\text{Val}_1-\text{Pro}_2^*-\text{Gly}_3-\text{Val}_4-\text{Gly}_5)_n-\text{Val}-\text{OMe}$

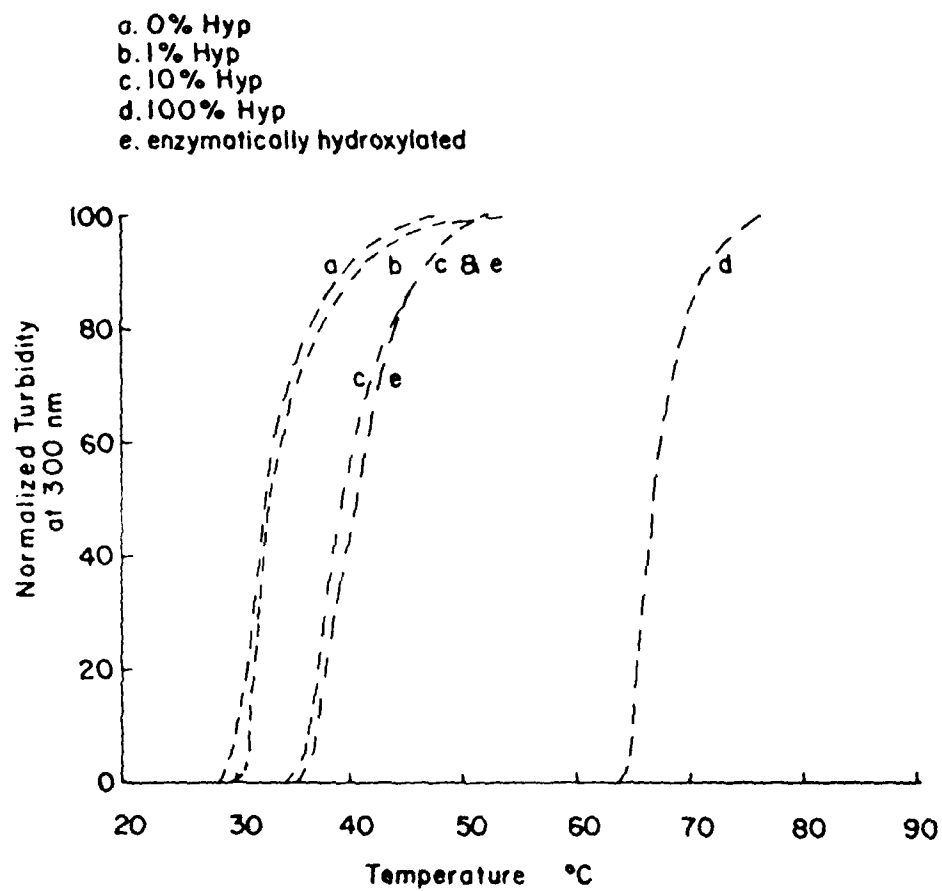


FIGURE 11

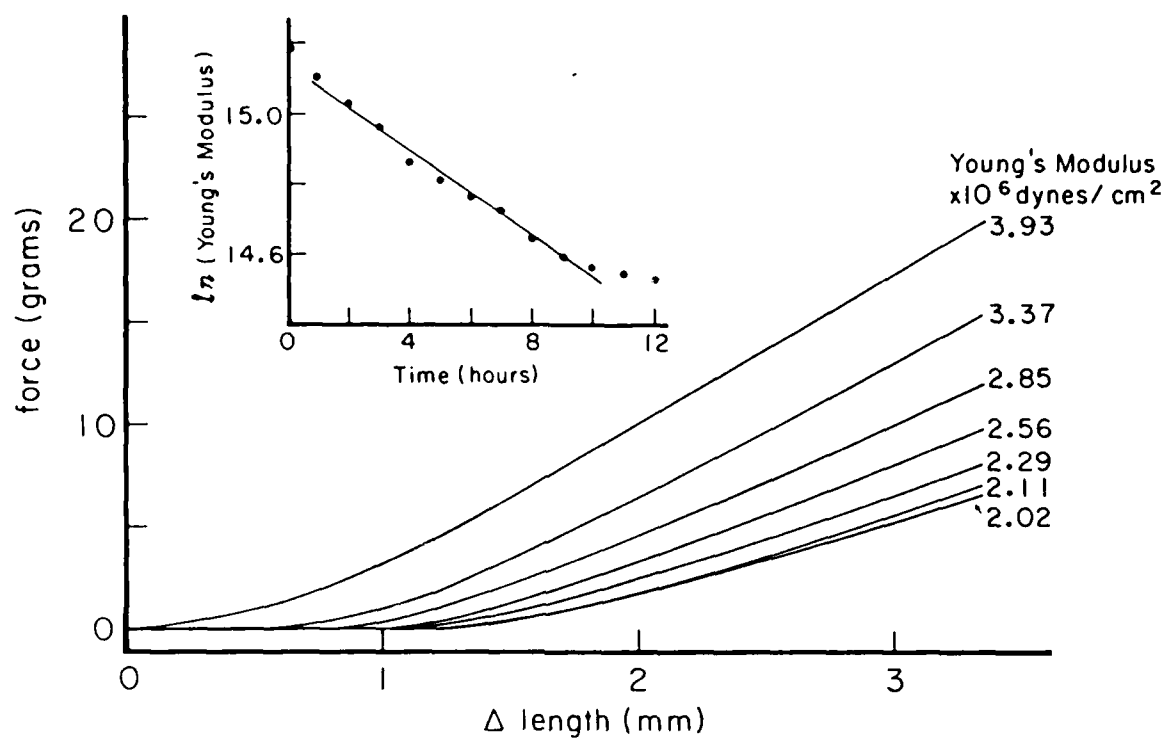


FIGURE 12

## Structural Transitions for Mechanochemical Coupling

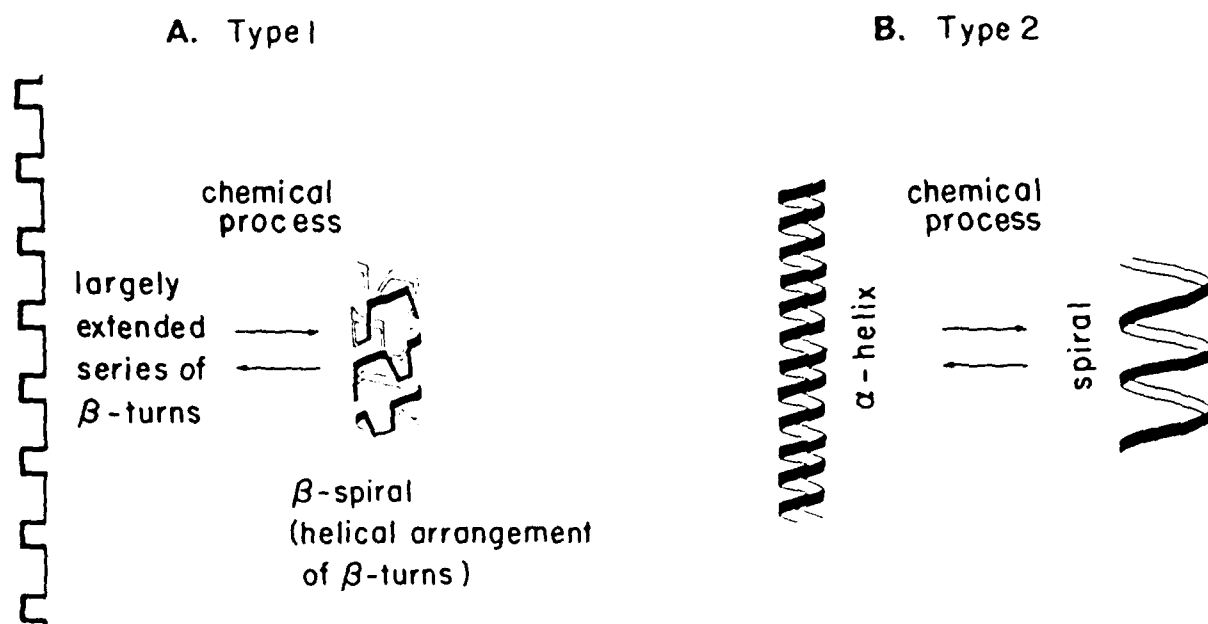


FIGURE 13

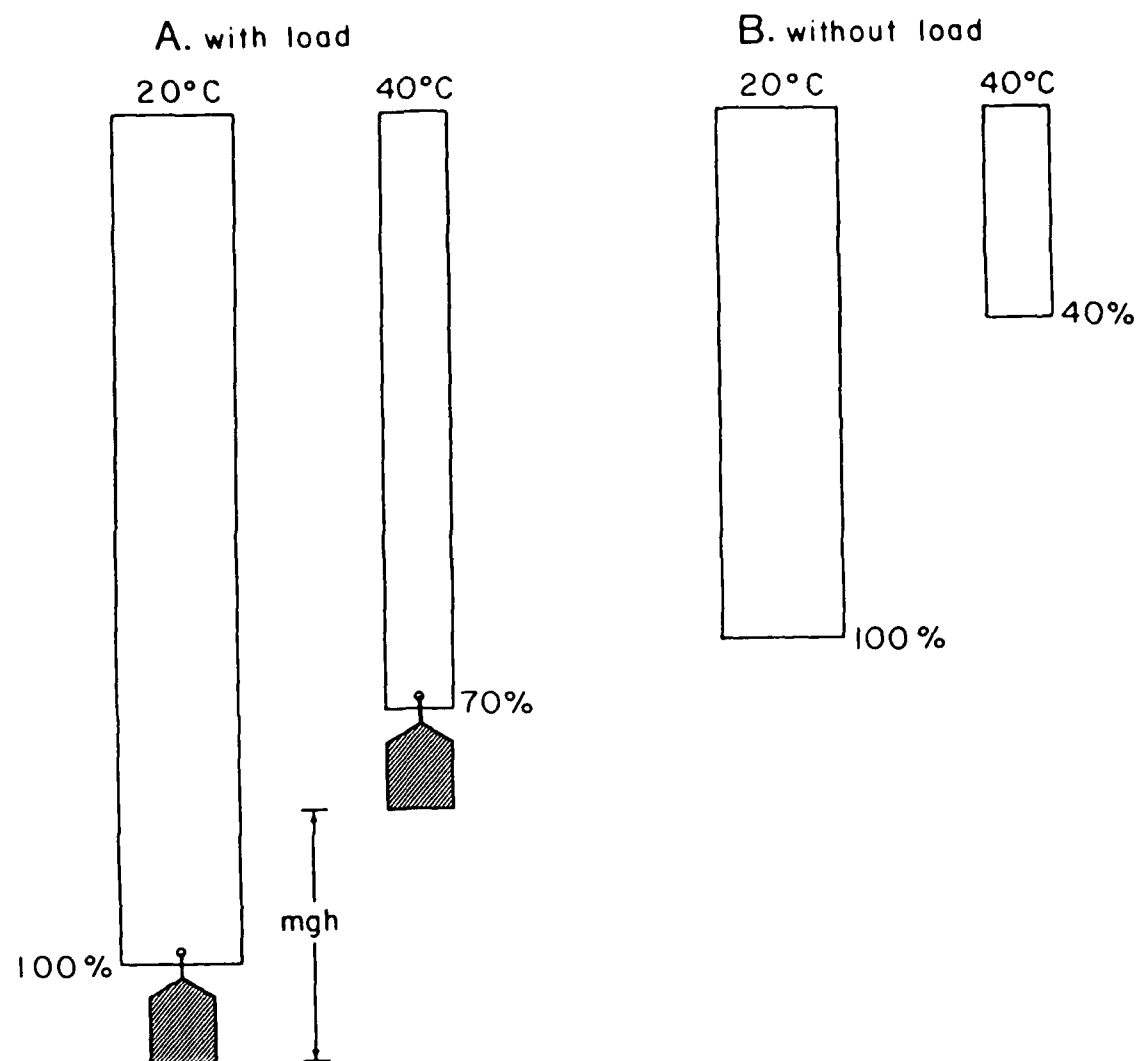


FIGURE 14



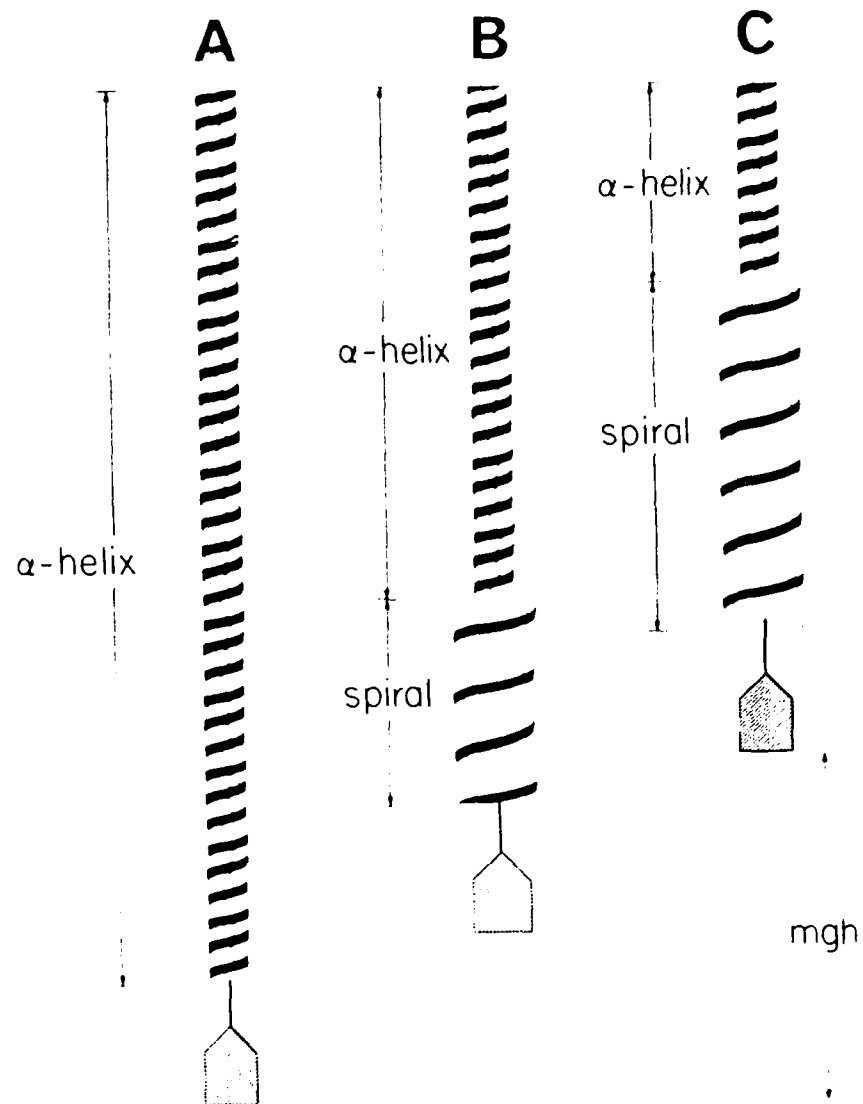


FIGURE 15

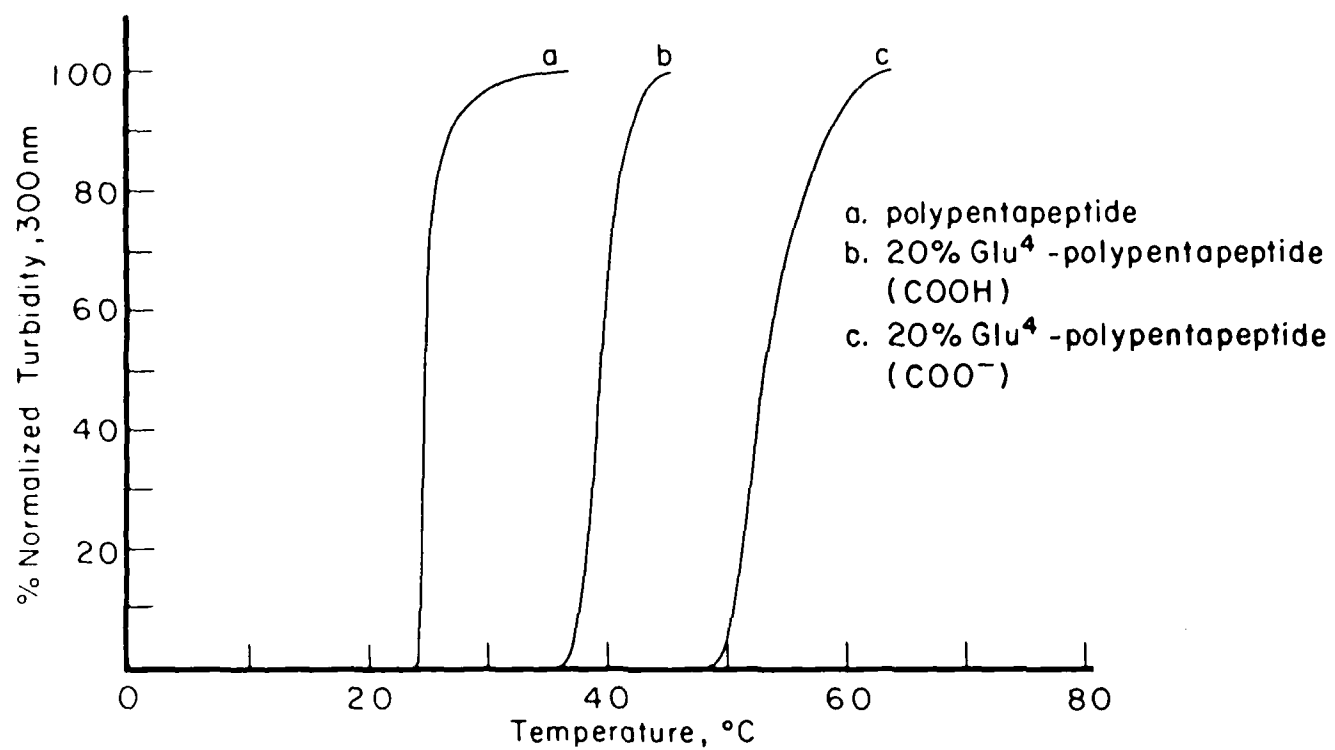


FIGURE 16

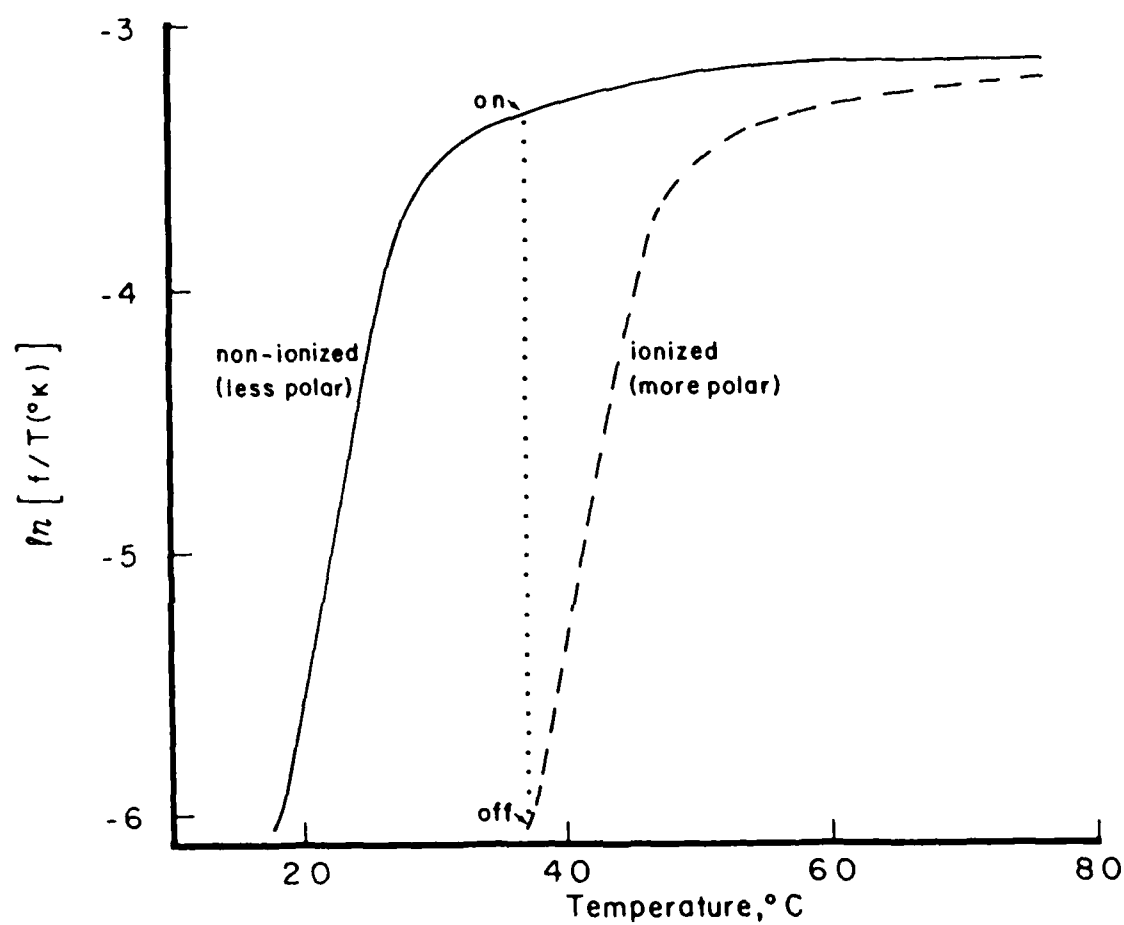


FIGURE 17

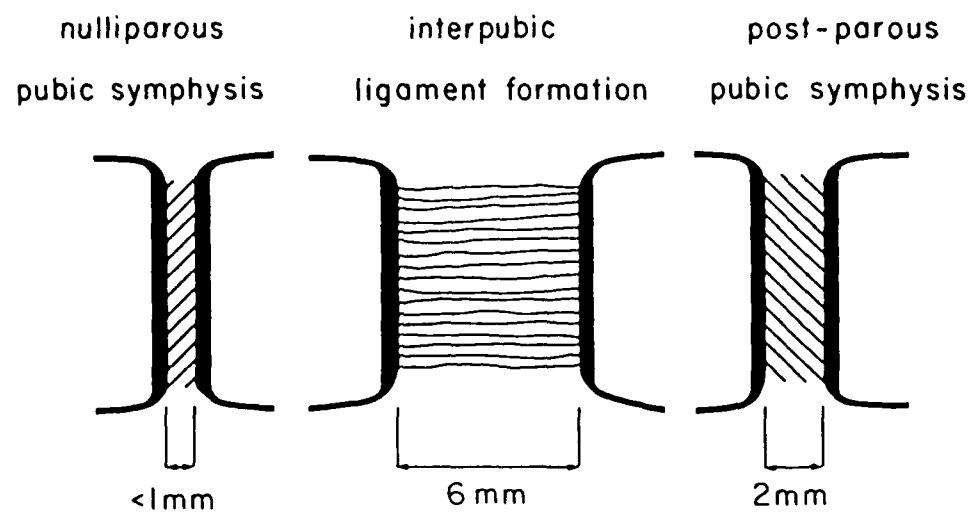


FIGURE 18

### Cell Migration: Pseudopod Formation

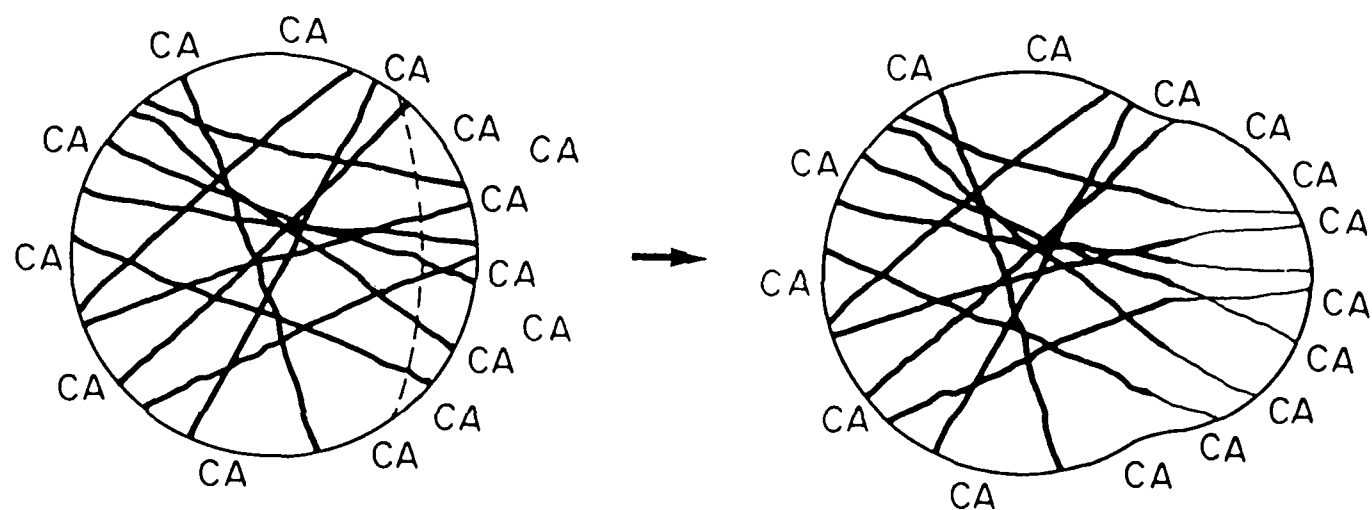


FIGURE 19

END

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